APPLICATION OF SOME METHODS FOR DETECTING ADULTERATION IN OLIVE OIL

By

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B.Sc. Agric. Sc. (Food Technology), Ain Shams University, 2000

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ABSTRACT

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This study aimed to maintain the high quality of olive oil by investigating some analytical methods as a powerful tool to determine extra virgin olive oil adulteration with relatively cheap seed and vegetable oils such as sunflower oil, corn oil and refined olive oil.

The analytical methods ranged from the familiar manual physical and chemical tests such as refractive index (RI) which gives a good idea about the degree of unsaturation of the oil under investigation, as well as its correlation with iodine value (IV); acid value (AV) as indication of free fatty acid content of the oil; peroxide value (PV) which determines the amount of primary oxidation products and UV absorbencies at 232 and 270 nm, that measure the formation of conjugated dienes and trienes, respectively due to the formation of secondary oxidation products.

The analytical methods extended to the more sophisticated instrumental methods of analysis such as chromatographic separation and determination techniques which involved gas chromatography (GC) with flame ionization detector (FID) in order to investigate the composition of the fatty acids of the oils under investigations.

Fourier Transform Infra Red (FTIR) spectroscopic determination technique was employed and applied as a potent, nondestructive and effective analytical tool to study its potency to investigate the functional groups with their relative absorbencies or transmittances according to their concentrations in samples and their characteristic fingerprints. FTIR spectroscopy was used also to determine extra virgin olive oil adulteration with sunflower, corn and refined olive oils in their binary admixtures at different concentrations of 0, 5, 10, 20, 30, 40, 50, 100%; w/w.

FTIR spectral data collected in MIR range 4000-400 cm^{-1} showed major peaks representing triglyceride functional groups which could be observed around 2925 cm^{-1} [C–H stretching (asymmetry)], 2854 cm^{-1} [C–H stretching (symmetry)], 1747 cm^{-1} [C=O stretching], 1463 cm^{-1} [C–H bending (scissoring)], 1238, 1163, 1118 and 1097 cm^{-1} [C–O stretching] and 722 cm^{-1} [C–H bending (rocking)]. A peak around 1653 cm^{-1} is attributed to C=C stretching (*cis*). The spectral region (1300-1000 cm^{-1}) which contains FTIR fingerprints of the used oils was found to be very useful in detecting extra virgin olive oil adulteration.

A band shift at 3005 cm^{-1}; assigned to C–H stretching vibration of *cis*- double bond (=C–H) characteristic to extra virgin olive oil, was observed at higher wavenumbers with increasing adulterant concentration which allowed the determination of adulteration of extra virgin olive oil.

The absorption intensity values of the spectral bands at 1163 cm^{-1} (assigned to C–O stretching vibration and CH_2 bending vibration) increased with increasing adulterant concentration.

There was a pronounced shift of the peak at 912 cm^{-1} (assigned to –HC=CH– of *cis*- double bond, bending out-of-plane) for extra virgin olive oil to higher wave numbers with increasing adulterant concentration.

Absorbance ratios (R1118/1097 and R1747/2925 cm^{-1}) decreased with increasing the concentrations of added adulterant oils (sunflower, corn and refined olive oils) with a fairly good linear relationship.

The spectral region selected between 1800-900 cm^{-1} mostly represented the combination of C–H bending, C=O stretching and C=C stretching and hence it was directly related to the unsaturated C=C bond. It played a very important role in the discriminant analysis.

In conclusion, FTIR spectroscopy proved its potency to detect extra virgin olive oil adulteration at 5% level of adulterant oils (sunflower, corn and refined olive oils) which is much lower than the limit at which there exists a threatening of adulteration of extra virgin olive oil.

KEY WORDS: Adulteration; extra virgin olive oil; FTIR spectroscopy; UV spectroscopy; GC analysis; sunflower oil; corn oil; refined olive oil.

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LIST OF ABBREVIATIONS

APCI	Atmospheric Pressure Chemical Ionization
APPI	Atmospheric Pressure Photospray Ionization
ATR	Attenuated Total Reflectance
AV	Acid Value
CEC	Capillary Electrochromatography
CL	Chemiluminescence
CLA	Conjugated Linoleic Acids
CO	Corn Oil
CVA	Canonical Variate Analysis
DEPT	Distortionless Enhancement by Polarization Transfer
DSC	Differential Scanning Calorimetry
DTGS	Deuterated Tri-Glycine Sulfate
ECN	Equivalent Carbon Number
ED	Energy-Dispersive
EEFM	Excitation–Emission Fluorescence Matrices
EEFS	Excitation–Emission Fluorescence Spectroscopy
ESI	Electrospray Ionization
EVOO	Extra Virgin Olive Oil
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
FID	Flame Ionization Detector
FIR	Far-IR
FTIR	Fourier Transform Infra Red
GC	Gas Chromatography
GC–FID	Gas Chromatography – Flame Ionization Detector
GC-MS	Gas Chromatography–Mass Spectrometry
GILS	Genetic Inverse Least Squares
GLC	Gas Liquid Chromatography
HPLC	High-Performance Liquid Chromatography

HPLC-APCI-MS	High-Performance Liquid Chromatography combined with Atmospheric Pressure Chemical Ionization coupled with Mass Spectrometry
HT-GC	High Temperature – Gas Chromatography
ID	Internal Diameter
IOOC	International Olive Oil Council
IV	Iodine Value
LC-MS	Liquid Chromatography with Mass Spectrometry
LDA	Linear Discriminant Analysis
MIR	Mid-infrared
MRI	Magnetic Resonance Imaging
MS	Mass Spectroscopy
MSC	Multiplicative Signal Correction
MUFA	Mono-unsaturated Fatty Acid
NIR	Near-infrared
NMR	Nuclear Magnetic Resonance
OPO	Olive–Pomace Oil
OSC	Orthogonal Signal Correction
PCA	Principal Component Analysis
PCR	Principal Component Regression
PDO	Protected Denomination of Origin
PLS	Partial Least Square
PLS-DA	Partial Least Square-Discriminant Analysis
PR	Pattern Recognition
PRESS	Prediction Residual Error Sum of Squares
PUFA	Poly-unsaturated Fatty Acid
PV	Peroxide Value
QC	Quality Control
QqTOF	Quadrupole Time-Of-Flight
R	Absorbance Ratio
ROO	Refined Olive Oil

RP-HPLC	Reversed Phase – High Performance Liquid Chromatography
SD	Standard Deviation
SEP	Standard Error of Prediction
SFA	Saturated Fatty Acid
SFC	Supercritical Fluid Chromatography
SFE	Super-critical Fluid Extraction
SIMCA	Soft Independent Modeling of Class Analogy
SO	Sunflower Oil
SPE	Solid Phase Extraction
SPME–MDGC	Solid Phase Microextraction coupled with Multidimensional Gas Chromatography
TAGs	Triacylglycerols
TG	Triglyceride
TH	Total Hydrocarbons
TLC	Thin-Layer Chromatography
ToFMS	Time of Flight Mass Spectrometry
TS	Total Sterols
TSFA	Total Saturated Fatty Acids
TSyF	Total Synchronous Fluorescence
TUFA	Total Unsaturated Fatty Acids
U-PCA	Unfold Principal Component Analysis
USFA	Unsaturated Fatty Acids
UV	Ultra Violet
VOO	Virgin Olive Oil
WD	Wavelength-Dispersive
XRF	X-Ray Fluorescence

1-INTRODUCTION

Olive oil, used since 4000 B.C. by the Mediterranean populations as a food, drug, and cosmetic, has been the object of numerous clinical and experimental studies in the last few decades, confirming its protective action. Because of increased scientific research, the recognition of virgin olive oil's biologic value has notably increased. In addition to olive oil's preventive action in the physiologic aging process in atherosclerosis and neoplastic development, it exerts protective action toward the skin with topical and dietary use **(Publio and Marzia, 2009).**

Virgin olive oil is one of the most used dressings and cooking fats in the Mediterranean countries. One of the most important characteristics of olive oils is the presence of a high content of oleic acid, which accounts for 60–80% of the total fatty acids (FA) and for approximately 90% of the mono-unsaturated fatty acids (MUFA). Poly-unsaturated fatty acids (PUFA), namely linoleic and linolenic acids, account for 5–8% of total fatty acids, and together with MUFA are considered to be nutritionally favorable **(Maggio et al., 2009).**

The high nutrition value of olive oil is mainly due to its high oleic acid content and low levels of free fatty acids, pigments, hydrocarbons and oxygenated compounds. Due to the high ratio of monounsaturated fatty acids to polyunsaturated fatty acids and to high levels of natural antioxidants (phenols and tocopherol); olive oil is very resistant to peroxidation (which is involved in free radicals formation which are highly toxic and detrimental to health). The world production of olive oil is ca. 3 million metric tons/annum. The olive fruit contains about 50% water, 20% oil, 20% carbohydrates (pectic, cellulosic and hemicellulosic substances), organic acids, pigments, phenolic compounds and minerals. 96–98% of the oil is found in the flesh (mesocarp) and skin (pericarp). Only 2–4% oil is found in the pit (endocarp). The common methods of olive oil extraction include physical or mechanical processes, chemical procedures or a combination of these methods **(Najafian et al., 2009).**

The sensory characteristics of bitterness and pungency are due to activation of taste receptors and trigeminal nerve endings associated with taste buds in fungiform papillae, sensitive to chemical stimuli. In virgin olive oils these sensations are related to the presence of phenolic compounds and can persist for rather long time after deglutition, showing a clear after-effect that can strongly vary among olive oils in intensity and duration and might affect consumer acceptance **(Esti et al., 2009).**

Recently, some pharmacological effects other than antioxidant capacity have been reported for olive oil phenols. (-)-oleocanthal, a component extracted from newly-pressed extra-virgin olive oil, possesses ibuprofen-like cyclooxygenases (COX-1 and COX-2) inhibitory ability; hydroxytyrosol and hydroxy-isochromans are inhibitors of platelet aggregation and oleuropein can form a non-covalent complex with amyloid-b (Ab) peptide or its oxidized form. All of these effects help to explain the benefits of olive oil in preventing cardiovascular diseases, cancer and Alzheimer's disease (AD). It was found that 13 olive oil phenols (caffeic acid, cinnamic acid, gallic acid, dihydroxybenzoic acid, *p*-hydroxybenzoic acid, benzoic acid, syringic acid, *p*-coumaric acid, oleuropein, verbascoside, pinoresinol, catechol, hydroxytyrosol) had been recorded in the comprehensive medicinal chemistry (CMC) data base which contains 8659 drugs; and the traditional Chinese medicine data base (TCMD) which contains 10458 components. Some pharmacological activities associated with anti-cancer, anti-cardiovascular diseases and anti-Alzheimer's disease were identified for caffeic acid (CNS exhilarant), oleuropein (vasodilator, coronary vasodilator, antispasmodic, antihypertensive, antiarrhythmic), *p*-coumaric acid (antihyperlipidaemia), verbascoside (anti-inflammatory, 5-lipoxidase inhibitor) and pinoresinol (cAMP phosphodiesterase inhibitor). With the rapid progress in pharmacological research on olive oil phenols, it can be expected that we will fully understand the health benefits of olive oil which is likely to be partially responsible for the health effects of Mediterranean diet in the not-very-distant future **(Yang et al., 2007).**

Virgin olive oil is highly appreciated because of its fine aroma, pleasant taste and health benefits. According to the International Olive Oil Council (IOOC, 2003) ‘‘virgin olive oil” is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil. Virgin olive oil, due to its high price, is a target for adulteration with low price/quality oils. Seed oils including corn, sunflower, rapeseed, soybean and walnut oils as well as low quality olive oil such as olive-pomace are commonly used for olive oil adulteration. Olive-pomace oil is produced through extraction of olive-pomace with toxic organic solvents and has significantly lower nutritive and price value in comparison to virgin olive oil. Adulteration is a major issue in the olive oil market. Detection is important for the protection of wealth and health of consumers. It should be noted here that olive oil is the major edible oil used in the Mediterranean coast by European Union consumers. Italians, Spaniards and Greeks are consuming 29, 32 and 47 grams per person per day, respectively. Various methods are used to detect adulteration spanning from classic wet chemistry to the more advanced chromatographic methods relying on quantification of fatty acids, triglycerols, sterols and hydrocarbons. Recently, spectroscopic techniques, which are rapid and easy to use, have also been used to detect adulteration **(Poulli et al., 2007).**

A lot of methods and limits were introduced into the International Olive Oil Council (IOOC) trade standard, into EC Regulation 2568/91 and into the Codex Alimentarius Standard for controlling product authenticity and quality **(Christopoulou et al., 2004).**

A weak chemiluminescence (CL) emission was observed in commercial Greek extra virgin olive oils and in refined seed oils such as sunflower oils as well as in corn oils with potassium superoxide in the aprotic solvent dimethoxyethylene. The light is produced by the oxidation of polyunsaturated fatty acid esters, such as linoleic or linolenic acid, and possibly energy transfer to fluorescent species contained in edible oils. On measuring the CL of mixtures of extra virgin olive oils with the cheaper refined seed oils, calibrations were produced which can be used for the determination of the adulteration of olive oils with seed oils down to 3%. Furthermore, depending on the kind of oils, “low” authenticity-CL-factors for olive oils (0.8–2.15μmol L^{-1} gallic acid) and “high” for seed oils (4.5–11.2μmol L^{-1} gallic acid) were calculated. For the evaluation of authenticity factors, gallic acid was used as reference because of its intense CL signals and its broad linear range extending from 1×10^{-6} to 1×10^{-2} mol L^{-1} **(Papadopoulos et al., 2002).**

The differentiation of virgin olive from olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils using total synchronous fluorescence (TSyF) spectra was carried out. TSyF spectra are acquired by varying the excitation wavelength in the region 250–720 nm and the wavelength interval ($\Delta\lambda$) in the region from 20 to 120 nm. It is shown that adulterants can be discriminated from virgin olive oil using a wavelength interval of 20 nm and excitation wavelength regions of 315–400, 315–392, 315–375, 315–365, 315–375 and 315–360 for olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils, respectively. 31 virgin olive oil mixtures with each potential adulterant were prepared at varying levels with emphasis at low concentrations. The partial least-squares regression model was used to quantify adulteration. This technique is useful for detection of olive-pomace, corn, sunflower, soybean, rapeseed and walnut oil in virgin olive oil at levels of 2.6%, 3.8%, 4.3%, 4.2%, 3.6%, and 13.8% (w/w), respectively **(Poulli et al., 2007).**

Adulteration of extra virgin olive oil with hazelnut oil is one of the most difficult to detect due to the similar composition of hazelnut and olive oils. The great concern existing nowadays about oil authentication has lead to the need of the development of new methodologies capable to detect fraud by adulteration, being hazelnut oil one of the most concerning adulterants. It has been proved that the proposed methods allow the correct detection and quantification of crude hazelnut oil in virgin and refined olive oils. The direct analysis of the oil samples by coupling headspace autosampling with mass spectroscopy (MS) detection, offers the advantages of rapidity and reliability. Within a practical point of view, the minimum adulteration levels reached by the proposed methods are low enough to permit the detection and quantification of adulterations in commercial olive oil **(Peña et al., 2005).**

The sterol composition of extra virgin olive oil is very characteristic and, thus, has become a helpful tool to detect adulterations with other vegetable oils. Special attention has been addressed to the separate determination of the free and esterified sterol fractions, since both have different compositions and can thus provide more precise information about the actual origin of the olive oil. In the case of admixtures with small amounts of hazelnut oil, this approach can be extremely useful, because the similarity between the fatty acid compositions of both oils hampers the detection of the fraud. A hyphenated chromatographic method was developed for a sensitive and precise determination of esterified sterols in olive oils by coupling the gas chromatograph with mass spectrometric detector **(Cercaci et al., 2003).**

Another innovative technique based on X-ray scattering applied to classify complex organic matrices of different vegetable oils. Vegetable oils from corn, canola, soybean, sunflower and olive (extra virgin and others) were analyzed and classifications were obtained using information from the scattered radiation **(Bortoleto et al., 2005).**

Fourier transform infrared (FTIR) spectroscopy was used as a rapid, nondestructive, authenticity measuring tool for edible oils, particularly for extra virgin olive oils. The presence of sunflower oil (as adulterant) in extra virgin olive oil can be detected through FTIR spectroscopy. Multivariate classification methods as discriminant analysis can be used to classify pure vs. adulterated olive oil samples successfully down to an adulteration level of 20mL of sunflower oil in 1 L of extra virgin olive oil. Multivariate quantification based on PLS successfully quantified the composition of sunflower oil, i.e. the degree of adulteration in the olive oil. Finally, discriminant analysis was used to successfully classify extra virgin olive oil and other vegetable oils **(Tay et al., 2002).**

AIM OF INVESTIGATION

The objective of the present study was to develop reliable and sensitive methods for detecting whether or not the extra virgin olive oil (EVOO) has been adulterated, and if so, with which oil is adulterated; and at what concentration?

This objective was achieved throughout the following fulfill:

- Preparing binary admixtures from EVOO with adulterant cheaper oils at various concentrations.
- The EVOO and adulterant oils individually and in their admixtures were exposed to applicable analytical determinations for developing a quantitative assay which accurately reflects the adulterant oil and the received level.

These applicable analytical determinations were as follows:

1- Evaluation of some quality indices and physicochemical parameters such as: refractive index, free fatty acid content, total unsaponifiable matters, peroxide value, iodine value, spectrophotometric characteristics in the ultraviolet region at 232 nm, 270 nm, ΔK and comparing the obtained data with those defined by IOOC and Codex Alimentarius Standard.

2- Application of gas chromatographic (GC) separation and determination technique for the identification of fatty acids.

3- Application of FTIR spectroscopic determination technique to explore its capabilities in detecting the adulteration of extra virgin olive oil by other edible oils such as sunflower, corn and refined olive oils to demonstrate the potential of this method in the detection of adulteration of extra virgin olive oil.

4- Deducing and developing the useful analytical assay which accurately reflects the adulteration of extra virgin olive oil and has the potential to quantify the amount of the adulterant oil.

2- REVIEW OF LITERATURE

2.1- Physicochemical Quality Parameters of Virgin Olive Oil

The study of the quality characteristics of virgin olive oil is of great interest to the local industrial sector, the international olive oil business, and the final consumer **(Salvador et al., 2001).**

Several physical and chemical tests have been proposed by several investigators for detecting adulteration of virgin olive oil as following:

Salvador et al. (2001) carried out a study on Cornicabra virgin olive oil from five successive crop seasons (from 1994/95 to 1998/99). The Cornicabra olive variety accounts for more than 14% of the olive oil produced in Spain. Free fatty acid (% as oleic acid) ranged between 0.08-0.55, peroxide value (meq O_2/Kg) ranged between 1.9-19.1, K_{232} ranged between 1.438-1.880 and K_{270} ranged between 0.099-0.229.

Gutiérrez et al. (2002) reported the following physicochemical parameters of extra virgin olive oil samples from three cultivars (Picual, Hojiblanca, and Arbequina); acidity (% as oleic acid) was 0.15; 0.20; 0.25, peroxide value (meq O_2/Kg) was 6.60; 4.30; 7.50, K_{232} was 1.41; 1.64; 1.93, and K_{270} was 0.11; 0.11; 0.11, respectively.

In this respect **Espínola et al. (2009)** investigated extra virgin olive oil from the same three olive cultivars (Picual, Hojiblanca, and Arbequina) and obtained the following physicochemical parameters; acidity (% as oleic acid) was 0.24; 0.37; 0.26, peroxide value (meq O_2/Kg) was 4.99; 7.15; 6.30, K_{232} was 1.54; 1.51; 1.65, K_{270} was 0.16; 0.16; 0.11, and ΔK was -0.004; -0.004; -0.003, for the above respective cultivars.

Ninfali et al. (2002) investigated some physicochemical properties of Italian and Spanish extra virgin olive oils, and reported the following parameters for these regions; acidity (% as oleic acid) was 0.36 for Italy; 0.37 for Spain, peroxide number (meq O_2/Kg) was 5.68 for Italy; 13.5 for Spain, K_{232} was 1.53 for Italy; 1.95 for Spain, and K_{270} was 0.14 for Italy; 0.14 for Spain.

However, **Cercaci et al. (2003)** investigated the Italian extra virgin olive oil, and found that; acidity (% $C_{18:1}$) was 0.28, peroxide value (meq O_2/kg) was 8.5, K_{270} was 0.11, K_{232} was 2.03 and $\Delta K < 0.01$.

Codex Alimentarius Standard (2003) established Codex standard for olive oils and olive-pomace oils, in which the following parameters were listed for extra virgin olive oil; free acidity (expressed as oleic acid) of not more than 0.8 gram per 100 gram; refractive index (at 20^{o}C) ranged between 1.4677-1.4705, iodine value (Wijs) ranged between 75-94, absorbency in UV at 270 nm; $K_{270} \leq 0.22$, absorbency in UV at 232 nm; $K_{232} \leq 2.50$; $\Delta K \leq 0.01$, peroxide value for virgin olive oils ≤ 20 milliequivalents of active oxygen/kg oil.

In 2005a, Egyptian Standards established Codex Standard for olive oils and olive-pomace oils; and the **International Olive Oil Council (IOOC, 2008)** established Trade Standard applying to olive oils and olive-pomace oils, in which the aforementioned parameters were listed for extra virgin olive oil having the same values of **Codex Alimentarius Standard (2003).**

2.2- Fatty Acid Composition of Virgin Olive Oil

The official organizations had established limits with regard to the content of fatty acid in olive oil performed by gas chromatography (GC). These limits are used for the discrimination between genuine olive oil and other vegetable oils **(Christopoulo et al., 2004).**

Pinelli et al. (2003) evaluated the fatty acid contents of 10 monocultivar virgin olive oils, typical of Tuscan, Italy to develop an initial database on Tuscan germplasm collection in order to establish the criteria and indicators useful to correlate the agronomic characteristics with quality and typicity of the olive oil. The following monocultivar olive oils were considered: Tondino (T), Frantoio (F), Mignolo Cerretano (MC), Rossellino (R), Leccione (L), Olivastra Seggianese (OS), Morchiaio (M), Leccio Maremmano (LM), Emilia (E) and Ginestrino (G). The investigated fatty acids composition with respect to their corresponding cultivars were as follows; palmitic: 14.6; 12.6; 14.3; 13.7; 9.76; 14.34; 13.05; 16.4; 14.0; 13.2, palmitoleic: 1.93; 0.65; 0.8; 1.50; 0.35; 1.43; 1.00; 1.06; 1.76; 1.26, margaric: 0.02; 0.05; 0.04; 0.04; 0.04; 0.04; 0.03; 0.04; 0.03; 0.03, margaroleic: 0.08; 0.10; 0.06; 0.10; 0.06; 0.07; 0.07; 0.07; 0.06; 0.08, stearic: 1.81; 2.04; 2.06; 1.75; 2.65; 2.08; 2.07; 2.17; 1.72; 1.98, oleic: 75.2; 76.1; 72.5; 74.6; 77.2; 72.4; 76.1; 59.0; 72.2; 73.6, linoleic: 5.40; 7.19; 8.91; 6.95; 8.63; 8.57; 6.46; 19.8; 9.19; 8.62, linolenic: 0.52; 0.66; 0.73; 0.69; 0.63; 0.55; 0.57; 0.67; 0.57; 0.73, arachidic: 0.27; 0.32; 0.33; 0.36; 0.41; 0.34; 0.35; 0.37; 0.31; 0.27, eicosenoic: 0.17; 0.33; 0.23; 0.33; 0.28; 0.22; 0.34; 0.43; 0.25; 0.20, SFA: 16.7; 15.0; 16.7; 15.9; 12.9; 16.8; 15.5; 19.0; 16.0; 15.5, PUFA: 5.92; 7.85; 9.64; 7.64; 9.26; 9.12; 7.03; 20.5; 9.76; 9.35, oleic/linoleic 13.9; 10.58; 8.14; 10.7; 8.94; 8.44; 11.8; 2.98; 7.85; 8.54, MUFA/SFA: 5.0; 5.7; 5.0; 5.3; 6.8; 4.9; 5.4; 4.3; 5.2; 5.4, MUFA/PUFA: 14.1; 10.8; 8.6; 11.0; 9.4; 9.1; 12.0; 3.95; 8.60; 9.00.

Aranda et al. (2004) investigated the fatty acids composition (%) of Cornicabra virgin olive oil variety samples from five successive crop seasons (from 1995/96 to 1999/2000) in Spain. The investigated fatty acids composition (%) were as follows; palmitic, $C_{16:0}$: 6.99-11.05, palmitoleic, $C_{16:1}$: 0.49-1.11, margaric $C_{17:0}$: 0.04-0.07, margoleic, $C_{17:1}$: 0.08-0.11, stearic, $C_{18:0}$: 2.61-4.43, oleic $C_{18:1}$: 76.5-82.5, linoleic, $C_{18:2}$: 3.07-6.62, linolenic, $C_{18:3}$: 0.48-0.95, arachidic, $C_{20:0}$: 0.28-0.62, gadoleic, $C_{20:1}$: 0.27-0.39, behenic, $C_{22:0}$: 0.11-0.21, saturated fatty acids, SFA: 11.40-15.13, monounsaturated fatty acids, MUFA: 78.0-83.6 and polyunsaturated fatty acids PUFA: 3.67-7.22.

International Olive Oil Council (IOOC, 2008) established the ranges of the fatty acids composition in olive oils performed by gas chromatography (% m/m methyl esters) as follows: myristic acid < 0.05, palmitic acid: 7.5-20.0, palmitoleic acid: 0.3-3.5, heptadecanoic acid < 0.3, heptadecenoic acid < 0.3, stearic acid: 0.5-5.0, oleic acid: 55.0-83.0, linoleic acid: 3.5-21.0, linolenic acid < 1.0, arachidic acid < 0.6, gadoleic acid (eicosenoic) < 0.4, behenic acid < 0.2 and lignoceric acid < 0.2.

Allalout et al. (2009) investigated the fatty acids composition (%) of virgin olive oil of three Spanish cultivars: Arbequina, Arbequina I-18 and Arbosana and one Greek cultivar: Koroneiki; grown in Northern Tunisia under irrigated high-density plantation system. The investigated fatty acids composition with respect to their corresponding cultivars were as follows; $C_{16:0}$: 17.57±0.60; 17.37±0.36; 17.78±1.06; 11.65±1.77, $C_{16:1}$: 2.41±0.85; 2.08±0.32; 2.12±0.08; 1.078±0.06, $C_{18:0}$: 1.88±0.02; 1.85±0.10; 2.07±0.44; 2.15±0.20, $C_{18:1}$: 58.82±2.75; 61.89±3.16; 64.79±0.96; 75.53±0.09, $C_{18:2}$: 12.93±2.67; 11.58±2.50; 12.09±0.12; 8.56±0.74, $C_{18:3}$: 0.63±0.12; 0.69±0.17; 0.54±0.29; 0.26±0.14, $C_{20:0}$: 0.40±0.04; 0.39±0.05; 0.33±0.11; 0.42±0.15, $C_{18:1}/C_{18:2}$: 4.63±0.74; 5.44±0.90; 5.36±0.03; 8.85±0.78, SFA: 19.86±0.54; 19.61±0.22; 20.18±1.61; 14.22±2.12, MUFA: 61.24±3.60; 63.97±3.48; 66.91±1.04; 76.61±0.15, PUFA: 13.56±2.55; 12.27±2.33; 12.63±0.41; 8.82±0.88.

2.3- Unsaponifiable Matter Composition of Virgin Olive Oil

Sterols, also called phytosterols, make up the greatest proportion of the unsaponifiable fraction of olive oil. The contents of these sterols in different olive oils are limited by regulations established by the European Union, the International Olive Oil Council, and the Codex Alimentarius of the FAO/WHO to control against fraud **(Casas et al., 2004).**

Salvador et al. (2001) carried out a study on Cornicabra virgin olive oil in Spain. The sterols compositions were as follows; cholesterol: 0.06-0.60, brassicasterol: 0.00-0.57, 24-methylenecholesterol: 0.00-0.31, campesterol: 3.82-4.64, campestanol: 0.00-0.43, stigmasterol: 0.38–1.75, Δ7-campesterol: 0.00–2.27, Δ5-23-stigmastadienol: 0.00–0.38, clerosterol: 0.53–1.05, β-sitosterol: 81.2–88.2, sitostanol: 0.00–1.27, Δ5-avenasterol: 3.34–10.97, Δ5-24-stigmastadienol: 0.26–1.09, Δ7-stigmastenol: 0.09–1.30, Δ7-avenasterol: 0.18–0.44, apparent β-sitosterol (%):91.7–94.9, total sterols (mg/kg): 1014–2055.

Phillips et al. (2002) evaluated the free and esterified sterols composition of edible oils and fats. For extra virgin olive oil, they reported the free and esterified sterols (as mg/100 g) as follows; sitosterol: free 105.5; esterified 27.1, campesterol: free 3.4; esterified 1.1, stigmasterol: free 0.9; esterified not detected, Δ5-avenasterol: free 15.2; esterified 6.6, sitostanol: free 0.9; esterified 0.9, campestanol: free 0.7; esterified not detected, cholesterol: free < 0.5; esterified < 0.5, the total free sterols were 162 and the total esterified sterols were 166.

International Olive Oil Council (IOOC, 2008) established the sterols composition in virgin olive oils (% total sterols) as follows: cholesterol < 0.5, brassicasterol < 0.1, campesterol < 4.0, stigmasterol < campesterol in edible oils, Δ-7-stigmastenol < 0.5, β-sitosterol + Δ-5-avenasterol + Δ-5-23-stigmastadienol + clerosterol + sitostanol + Δ 5-24-stigmastadienol > 93.0, total sterol content ≥ 1000 (mg/Kg).

Ben Temime et al. (2008) studied the sterol profile of Tunisian virgin olive oils produced from Chétoui cultivar. Olive fruits were collected from ten distinct farms in the north of Tunisia during crop seasons 2003/2004 and 2004/2005. The investigated sterols composition (%) were in ranges as follows; cholesterol: 0.10-0.24, 24-methylenecholesterol: 0.10-0.28, campesterol: 2.45-2.86, campestanol: 0.07-0.14, stigmasterol: 1.10-4.93, clerosterol: 0.92-1.10, β-sitosterol: 76.80-84.40, sitostanol: 0.33-0.67, Δ5-avenasterol: 6.31-16.02, Δ5,24-stigmastadienol: 0.56-0.86, Δ7-stigmastenol: 0.09-0.24, Δ7-avenasterol: 0.23-0.49, apparent β-sitosterol: 91.77-95.12.

2.4- Detection of Adulteration of Virgin Olive Oil by DSC

Differential Scanning Calorimetry (DSC) monitors the changes of physical or chemical properties of a material as a function of temperature by detecting the heat changes associated with phase transitions such as crystallization and glass transition. DSC compares the rate of heat flow of a sample to that of an inert reference material as both are heated or cooled. DSC thermograms are characterized by endothermic and/or exothermic peaks whose area is proportional to the enthalpy gained or lost by the material undergoing phase transition. The phase transitions take place over a specific temperature range depending on the sample compositions and physical properties **(Vittadini et al., 2003).**

Chiavaro et al. (2008a) reported that Differential Scanning Calorimetry (DSC) has advantages over the more classical detection methods, as it is rapid and does not require sample preparation or solvent utilization. The application of DSC to the analysis and characterization of oils and fats for the determination of solid fat content, crystallization and melting profiles, enthalpy of transitions and polymorphic forms, is well known and reviewed. DSC application has also been suggested as a valuable tool for characterization of oils from vegetable sources and thermal parameters have been reported to correlate well with chemical parameters obtained with standard methods.

They also used DSC as a potential tool for discrimination of olive oil commercial categories. It was able to differentiate monovarietal virgin olive oils based on temperature (onset and transition range) of crystallization and melting profiles, which were well correlated with oleic and linoleic acid contents. A good relation was reported between thermal properties and major (triacylglycerols and fatty acids) and minor components (free fatty acids, diacylglycerols, and primary and secondary oxidation products) of monovarietal extra virgin olive oils for both cooling and heating profiles that were resolved into the constituent peaks and related to specific triacylglycerol (TAG) species. DSC application upon cooling and heating also appeared very promising in discriminating among oil samples from olives of different cultivars and/or harvesting periods.

DSC thermograms of five commercial categories of olive oils (extra virgin olive oil, olive oil, refined olive oil, olive-pomace oil and refined olive-pomace oil) were performed in both cooling and heating regimes. Overlapping transitions were resolved by deconvolution analysis and all thermal properties were related to major (triacylglycerols, total fatty acids) and minor (diacylglycerols, lipid oxidation products) chemical components. All oils showed two well distinguishable exothermic events upon cooling. Crystallization enthalpies were significantly lower in olive oils due to a more ordered crystal structure, which may be related to the higher triolein content. Pomace oils exhibited a significantly higher crystallization onset temperature and a larger transition range, possibly associated to the higher amount of diacylglycerols. Heating thermograms were more complex: all oils exhibited complex exo- and endothermic transitions that could differentiate samples especially with respect to the highest temperature endotherm. The evaluation of the cooling thermal properties appeared promising for discrimination between olive and olive-pomace oils. In particular, T_{on} and T_{off} of the crystallization showed significant differences among different commercial categories, while enthalpy could differentiate between olive (olive oil and refined olive oil) from olive-pomace oils (olive-pomace oil and refined olive-pomace oil). Preliminary results suggested that both cooling and heating thermograms obtained by means of DSC was useful for discriminating among olive oils of different commercial categories **(Chiavaro et al., 2008a).**

Chiavaro et al. (2008b) in another assay demonstrated the evaluation of potential application of differential scanning calorimetry (DSC) to verify adulteration of extra virgin olive oil with refined hazelnut oil. Extra virgin olive oil and hazelnut oil were characterized by significantly different cooling and heating DSC thermal profiles. The characterizing overall thermal properties (enthalpy, T_{on} and temperature range) obtained from the heating thermograms of pure oils and their admixtures, from the beginning of the exothermic to the end of the endothermic events, were recorded and organized. Enthalpy value of the total heating transition was calculated by subtracting the exothermic enthalpy from the endothermic peak enthalpies. Extra virgin olive oil displayed significantly lower heating enthalpy than did hazelnut oil and the admixtures. Enthalpy of the overall heating transition did not significantly change with 5–30% hazelnut oil addition. A significant increase was observed at 40% hazelnut oil, as the exothermic event disappeared. A more ordered crystal structure, having TAG chains more compactly associated that completely crystallized upon cooling, may be formed upon adding hazelnut oil, thus requiring higher energy to melt. T_{on} of the total heating event significantly shifted toward higher temperature, starting from 30% of hazelnut oil added, as the first exothermic event shifted toward higher temperature and disappeared at 40% hazelnut oil. Range of transition also significantly narrowed as the minor endothermic peak shifted toward lower temperature. The increase of higher unsaturated lipid fractions, and the consistent decrease of the more saturated ones, may both be responsible for T_{on} shifting and narrowing of transition, making the major endotherm more similar to the hazelnut oil line shape. It was concluded that the addition of hazelnut oil significantly enhanced crystallization enthalpy (at hazelnut oil concentration ≥ 20%) and shifted the transition towards lower temperatures (at hazelnut oil concentration ≥ 5%). Line shape of heating thermograms of extra virgin olive oil was significantly altered by hazelnut oil addition: a characteristic exothermic event originated at -27 ^{o}C in extra virgin olive oil and progressively disappeared with increasing hazelnut oil content, while the major endothermic peak at -3.5 ^{o}C broadened (at hazelnut oil concetration ≥ 40%) and the minor endothermic peak at 8 ^{o}C shifted toward lower temperatures (at hazelnut oil concentration ≥ 5%). The lowest percentage of hazelnut oil that could be detected by DSC was not completely established by preliminary results. However, hazelnut oil addition to extra virgin olive oil at economically remunerative levels (20–40%) was clearly detectable by DSC analysis.

2.5- Detection of Adulteration of Virgin Olive Oil by Hyphenated Chromatographic Separation and Determination Techniques

Hyphenated techniques for chromatographic detection have been widely used. The hyphenated techniques provide a synergy where the combination is better than either technique alone. GC-MS, GC-FTIR, GC-AED, HPLC-NMR are the most widely used hyphenated technique. In all of the many forms of chromatography, detection is an inherently important final step. The type of detection can aid in the analysis by gathering information that can be used to identify the peaks seen. There can be many peaks that elute from the column of a gas, liquid, or supercritical-fluid chromatograph. Certain detectors are in fact spectrometers that examine each peak for specific information on its identity. Hyphenated techniques deal with this use of spectrometers as the tail-end detector in chromatography. Other separation techniques, such as field-flow fractionation or capillary electrophoresis, differ in their separation mechanisms, but as far as coupling to spectrometers behave like one of these three types of chromatography. If the analysis is the separation of many common complex mixtures, then many peaks can be either partially or fully resolved **(Gauglitz and Vo-Dinh, 2003).**

The same authors added that the simplest and least expensive, and therefore the commonest, chromatographic detectors only yield a response for a peak, with little diagnostic power to identify it other than the retention time. The flame-ionization detector (FID) in GC and the refractive index (RI) or single-wavelength UV absorbance and fluorescence detectors in HPLC or SFC are good examples of simple, widely-used detectors of this type. This is not a limitation for routine analyses, such as those used for product quality control or process monitoring. For complex mixtures or for the situation where a problem has been identified and its causes need to be determined, chromatography with simple detectors is woefully insufficient. The hyphenated techniques, however, are ideal tools in many of these situations.

Detection of adulteration of virgin olive oil by hyphenated chromatography was investigated by several investigators as following:

Abidi (2001) reported chromatographic methods for the analysis of plant sterols in various sample matrices with emphasis on vegetable oils. The principal themes of the review highlight the development and application of chromatographic techniques for the isolation, purification, separation and detection of the title compounds. The review also covers specific analyses of natural/synthetic standard mixtures to shed light on potential applicability in plant sample assays. Examples of combined chromatographic techniques linked in tandem for the analysis of complex samples are included. Apparently, the separation power of capillary electrochromatography (CEC) is greater than high performance liquid chromatography (HPLC) which, in turn, is much greater than supercritical fluid chromatography (SFC). Gas chromatography (GC) appears to have greater ability (or comparable) to resolve complex mixtures than CEC despite superior analyte selectivity (and suitability for thermally labile analytes) of the latter. Analytical precision and sensitivity of the various techniques currently available for sterol assays seems to follow the order: GC > HPLC > SFC > CEC. The sensitivity order may vary depending on sterol structures (e.g. fluorescent labels) and detectors coupled to chromatographic instruments. While GC, HPLC, SFC and CEC (in its developmental phase) are individually meritorious for specific applications, GC–FID (or MS) is considered to be the method of choice for practical analyses of plant sterols in foods and vegetable oils.

Tasioula-Margari and Okogeri (2001) described a simple procedure for the simultaneous extraction and HPLC determination of phenols and tocopherols in virgin olive oil. The extraction was carried out using methanol and an isopropanol-methanol mixture. Separation was achieved on a reversed phase C18 column with acetic acid/water-methanol-acetonitrile-isopropanol mixture under gradient elution. Detection was accomplished with UV detection at λ=280 nm. Using this method, simple and complex phenols as well as α-tocopherol can be determined in one run. Phenolic compounds were totally extracted whereas an average recovery of 80% was achieved for α-tocopherol. Validation of method is reported for olive oil samples with varying concentrations of α-tocopherol and phenolic compounds.

Gordon et al. (2001) investigated the polar fraction from virgin olive oil and pressed hazelnut oil by HPLC. The chromatograms of the polar components in the two oils showed marked differences. Six commercial samples of pressed hazelnut oil and 12 samples of blended virgin olive oil were analyzed. The phenolic content of the pressed hazelnut oil samples was 161±6 mg/Kg. Inspection of the chromatograms showed that the pressed hazelnut oil extracts contained a component that eluted in a region of the chromatogram that was clear in the olive oil samples, and consequently this component could be used to detect adulteration of virgin olive oil by pressed hazelnut oil. The component had a relative retention time of 0.9 relative to 4-hydroxybenzoic acid added to the oil as an internal standard. Analysis of blends of oils showed that adulteration of virgin olive oil by commercial pressed hazelnut oil could be detected at a level of about 2.5%.

Jakab et al. (2002) evaluated different vegetable oil samples (almond, avocado, corngerm, grapeseed, linseed, olive, peanut, pumpkin seed, soybean, sunflower, walnut, wheat germ) using high-performance liquid chromatography–atmospheric pressure chemical ionization-mass spectrometry (HPLC-APCI-MS). A gradient elution technique was applied using acetone–acetonitrile eluent systems on an ODS column (Purospher, RP-18e, 12534 mm, 5 mm). Identification of triacylglycerols (TAGs) was based on the pseudomolecular ion $[M+1]^+$ and the diacylglycerol fragments. The positional isomers of triacylglycerol were identified from the relative intensities of the $[M\text{-}RCO_2]^+$ fragments. Linear discriminant analysis (LDA) as a common multivariate mathematical–statistical calculation was successfully used to distinguish the oils based on their TAG composition. LDA showed that 97.6% of the samples were classified correctly. It can be concluded that HPLC combined with APCI-MS is a suitable tool to analyze triacylglycerols present in different plant oils. HPLC–APCI-MS combined with linear discriminant analysis proved to be a suitable method for the discrimination of different oils according to their TAG composition.

Phillips et al. (2002) determined the free and esterified sterol concentrations in 31 edible oils and fats, including individual values for sitosterol, campesterol, stigmasterol, brassicasterol, Δ^5-avenasterol, sitostanol, campestanol, and cholesterol. Free and esterified sterols were separated by solid-phase extraction (SPE), saponified, and quantified as trimethylsilyl ether derivatives using capillary gas–liquid chromatography. Considerable variability in the proportion of free and esterified sterols was observed among different oils and fats, with free sterols ranging from 32 to 94% of total sterols. Refining or hydrogenation tended to decrease total sterols and increase esterified sterols. Differences in total phytosterol content and the proportion of free and esterified sterols also were evident for different samples of some oils. Such variability in phytosterol composition likely reflects differences in processing, growing season, or variety of a particular plant source.

Pinelli et al. (2003) found that virgin olive oil is consumed unrefined and rich in important molecules, such as minor polar compounds (hydroxytyrosol, tyrosol, secoiridoids and flavonoids) and fatty acids. These molecules not only influence the sensorial properties of both olives and virgin oil but they are also important markers for typicity, biodiversity and quality determination of this product. They evaluated the minor polar compound and fatty acid contents of 10 monocultivar virgin olive oils, typical of Tuscany, in order to have better knowledge about the quali-quantitative profiles of these compounds in samples obtained from both the same collecting season and same processing technique. Quali-quantitative analysis (performed by HPLC/DAD, HPLC/MS and GC) could be a useful tool to better correlate the typicity of the virgin olive oil with its minor polar compound and fatty acid pattern.

Dourtoglou et al. (2003) used the principal component analysis (PCA) to establish a new method for the detection of olive oil adulteration. The data set composed of values obtained from the determination of the mole percentage of total FA and their regiospecific distribution in position 1 and 3 in TG of oils (pure or mixtures) by GC determination of sn-1,3 distribution of FA in TAG by butyl ester formation using analysis, was subjected to PCA. In this work a single and easily applied technique like GC, coupled with a statistical tool (DA) has been introduced for the determination of any adulteration of olive oil. The proposed method can be divided in three distinguishable parts. The first and second parts are the determination of the total FA as FAME and as sn-1,3 fatty acid butyl esters (FABE), respectively. The third part consists of a data measured on a total of 20 oils (10 pure oils and 10 mixed oils). The pure oils used were corn, soybean, sunflower, cottonseed, and olive oil, and the mixed oils were made with olive oil and soybean oil (95:5, 50:50, vol/vol) and with olive oil and each of cottonseed oil; corn oil; sunflower oil (95:5, 50:50, 25:75, vol/vol). With this proposed method using a regiospecific lipase (1,3- specific lipase from *Mucor miehei*) it was possible to distinguish mixtures of olive oil with seed oils even at a level of 5%. It was also possible to predict the seed origin of the oil used for the adulteration. Additionally, many samples can be analyzed in a short time.

Laboratories dedicated for detection of adulteration can establish a data library and introduce new cases to it as they appear. Then this library can instantly give information on possible adulteration by simple incorporation of the GC results of the suspected oil into the data matrix. The used samples: corn, soybean, sunflower, cottonseed, almond and olive oils. The first is the blending of virgin olive oils with olive oils of lower grade as refined olive oil **(Dourtoglou et al., 2003).**

Cercaci et al. (2003) reported that the sterol composition of extra virgin olive oil is very characteristic and, thus, has become a helpful tool to detect adulterations with other vegetable oils. Special attention has been addressed to the separate determination of the free and esterified sterol fractions, since both have different compositions and can thus provide more precise information about the actual origin of the olive oil. In the case of admixtures with small amounts of hazelnut oil, this approach can be extremely useful, because the similarity between the fatty acid compositions of both oils hampers the detection of the fraud. A hyphenated chromatographic method was developed for a precise determination of esterified sterols in olive oils. The oil was subjected to silica solid-phase extraction (SPE) fractionation, cold saponification of the collected fraction and purification on silica TLC. The unsaponifiable fraction was fractionated on silica TLC, The sterol band was then extracted, silylated and injected into GC. The method was tested on extra virgin olive oil; good sterol recoveries and repeatability were obtained. The results were compared with another method, which has a different sample preparation sequence (silica column chromatography, hot saponification and silica TLC). Similar results were achieved with both methods; however, the SPE–cold saponification–TLC–capillary GC was faster, required less solvent and prevented sterol decomposition.

The SPE-method was applied to an admixture with 10% of hazelnut oil and to a screening of 11 oils (husk oil, virgin and refined olive oils) from different Mediterranean countries. The results presented in this work confirm the importance of the study of the different sterol fractions (esterified, non-esterified and total) of the olive oils, which could be a useful source of information for detection of adulterations in this sector **(Cercaci et al., 2003)**.

Gamazo-Vázquez et al. (2003) described a quality control method for the detection of contamination of olive oil by seed oil, such as may occur as the result of carryover in bottling lines, with the aim of diagnosing if cross-contamination has occurred and by how much. The method is based on gas chromatography of the methyl esters of the oil components, with detection by mass spectrometry (GC-MSD). In the example presented (contamination by sunflower seed oil), it was identified the optimal discriminatory parameter, which was the ratio between the peak areas of the oleic and linoleic derivatives in chromatograms obtained using full-scan MS between 35 and 350 amu; this ratio would allow diagnosis of contamination of olive oil by sunflower seed oil at least at the 1% level with >95% certainty in bottling plants. It is important that each bottling plant establish its own reference sample collection and identify its optimal discriminant parameters following the proposed procedures.

Park and Lee (2003) studied the separation of TG in edible vegetable oils by a wide range of techniques such as gas chromatography

(GC), liquid chromatography (LC), super- and sub-critical fluid chromatography (SFC and SubFC), and capillary electrochromatography (CEC). The most common approach to the analysis of TG is to release free fatty acid (FA) and perform GC or GC-mass spectrometry (MS) after derivatization, for example, methylation. This approach does not identify actual intact TG molecular species, but only determines the percentage of individual FA present in the total TG fraction.

However, the authors developed the detection of adulterated oils based on TG compositions by high temperature (HT)-GC. They obtained the TG profiles of selected vegetable oils by HT-GC. Then, detection of adulterated olive oil mixed with soybean oil was investigated by using TG profiles and principal components analysis (PCA).

Zabaras and Gordon (2004) described an improved method for the detection of pressed hazelnut oil in admixtures with virgin olive oil by analysis of polar components. The method, which is based on the SPE-based isolation of the polar fraction followed by RP-HPLC analysis with UV detection, is able to detect virgin olive oil adulterated with pressed hazelnut oil at levels as low as 5% with accuracy (90.0±4.2% recovery of internal standard), good reproducibility (4.7% RSD) and linearity (R^2: 0.9982 over the 5–40% adulteration range). Although not very rapid and not quantitative, the developed method could be used, on its own or in combination with other techniques, for the qualitative detection of adulteration of virgin olive oils adulterated to low levels (down to 5%) with most pressed hazelnut oils. The method was only 82% successful on average in the ring test with five independent laboratories, but this success rate could be improved by provision of training samples.

Christopoulou et al. (2004) investigated the determination of fatty acids and triglycerides in the detection of adulteration of olive oil with certain vegetable oils. They found that the use of the established limits for the fatty acids content could be useful for the detection of fraud of an olive oil with the following vegetable oils: soybean, walnut, canola, rapeseed, peanut and mustard, even at levels of adulteration below 5%.

However these could not be used to detect percentages lower than or equal to 5% of: sunflower, cotton, corn, sesame, safflower, hazelnut and almond oils in mixtures with olive oil. The parameter ΔECN^{42} is a very useful and effective tool in the detection of the presence of the most common vegetable oils. More specifically the established limit for the ΔECN^{42} in olive oil was satisfactory for the detection of adulteration of an olive oil with the following vegetable oils: soybean, cotton, corn, walnut, sesame, safflower, canola and rapeseed. The use of this limit allowed the detection of even very low levels of adulteration. The established limits for the ΔECN^{42} was not satisfactory for detecting percentages lower than or equal to 5% of: hazelnut, almond, peanut and mustard oils in mixtures with olive oil.

The parameters $(LLL/ECN^{42})*100$, ECN^{46}/LLL and $(ECN^{44} + ECN^{46})/LLL$, which are based on the differences in triglyceride and fatty acid composition between the olive oil and vegetable oils; could be used as a discriminator factor between the olive oil and the eight of the examined vegetable oils: sunflower, soybean, cotton, corn, walnut, sesame, safflower and canola. However, the use of these parameters was not satisfactory for detecting percentages lower than or equal to 5% of: rapeseed, hazelnut, almond, peanut and mustard oils in mixtures with olive oil. No single one of the official parameters or the proposed ones could detect the presence of percentages lower than or equal to 5% of: hazelnut and almond oils in olive oil since the fatty acid and triglyceride compositions of these oils are very similar to that of olive oil. The detection of hazelnut and almond oils in olive oils is very difficult issue and more research is needed **(Christopoulou et al., 2004).**

Aranda et al. (2004) observed that the main triglycerides (TG) found in the Cornicabra virgin olive oil variety samples analyzed were OOO, SOL + POO, OLO + LnPP and OLA + SOO as expected from the high oleic acid and low linoleic and linolenic acid contents for both the total and *Sn-2*-position fatty acids (FA). These accounted for more than 85% of the total HPLC chromatogram peak area concentrations of most of the TGs and FAs presented highly significant statistical differences among the four Spanish varieties studied. Principal component analysis and discriminant analysis (PCA and DA) suggested that the TG variables are more suitable than total and *2*-position FAs. For optimum classification of the commercial samples analyzed finally; PCA and DA showed that there were several combinations of TGs and total FAs variables which can be selected for satisfactory classification of the four Spanish virgin olive oil studied, guaranteeing 90% + correct classification.

Alves et al. (2005) evaluated the sterol compositions (GLC/FID/capillary column) of monovarietal olive oils (51 samples) from the most important cultivars of northeastern Portugal (Cvs. Cobrançosa, Madural and Verdeal Transmontana) and 27 commercial samples of olive oils with protected denomination of origin (PDO) from the same region and cultivars. β-sitosterol, Δ^5-avenasterol and campesterol were the most representative sterols. Cholesterol, stigmasterol, clerosterol and Δ^7-stigmastenol were also found in all samples. All studied samples respected EC Regulation No. 2568, and in all cases total sterols were remarkably higher than the minimum limit set by legislation, ranging from 2003 to 2682 mg/kg. Results were analyzed with the help of several statistical techniques for approximate classification of monovarietal and PDO olive oils. They investigated the possibility to evaluate the authenticity of PDO olive oils for the characterization of monovarietal olive oils according to their sterol composition. The approaches used in their work were likely to be applied successfully on the classification and/or authenticity evaluation of many commercial materials that are blends.

Hajimahmoodi et al. (2005) demonstrated that the partial least squares (PLS) modeling method and gas-chromatographic fatty-acid fingerprints were considered as a method for the simultaneous determination of cottonseed, olive, soybean and sunflower edible oil mixtures. In this work, two sets of three- and four-component combinations of oils were prepared, hydrolyzed and the obtained free fatty acids analyzed by gas chromatography (GC) without any further derivatization. The normalized percentages of the myristic, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids were chromatographically measured in samples and used for constructing calibration matrix. The cross-validation method was used to select the number of factors and the proposed methods were validated by using two sets of synthetic oil mixture samples. The relative standard error for each oil in mixture samples was less than 10%. This approach allowed predicting the composition of unknown oil mixtures, and determining possible adulteration in each of the four edible oils.

Nagy et al. (2005) described the application of an reversed phase high performance liquid chromatography coupled with mass spectrometry (RP-HPLC MS) method in combination with chemometric evaluation for efficient characterization of extra virgin olive oils. The advantages of the proposed method are (a) simplicity: no derivatization or sample purification is needed; (b) speed: separation occurs within 20 min; (c) robustness: repeatability of retention times is 1%, repeatability of signal heights is 10%; (d) sensitivity: more than 50 compounds were detected in the methanolic solution of olive oil; (e) price: no chemicals or consumables tools are needed for sample preparation, in addition solvent consumption is lower than compared to monolithic columns while providing similar elution times. The main advantages of the combination of chemometric evaluation with HPLC–MS are threefold: first, stepwise discriminant function analysis can be used to select the most discriminative variables detected during the HPLC–MS experiment. Second, application of linear Discriminant analysis in combination with HPLC–MS can be used to classify extra virgin olive oil samples, e.g. originating from different cultivars. Third, cross-validation is an invaluable tool to give confidence in the obtained results and to indicate the predictive potential of the HPLC–MS technique developed. These advantages make this method a practical choice both for research and for quality control purposes.

Cunha and Oliveira (2006) applied an HPLC procedure for the determination of triacylglycerol (TAG) profile in vegetable oils. A total of 15 peaks were separated, identified, and quantified based on the relative percentage peak area. After validation the methodology was applied to eight vegetable oils including olive oil in a total of 52 samples. The proposed method appears to be an adequate method for quality control and a useful tool for authenticity issues.

Flores et al. (2006a) studied the adulteration of olive oil with hazelnut oil by online coupled high performance liquid chromatographic and gas chromatographic analysis of filbertone. The optimization of the interface performance in the on-line coupling of reversed phase liquid chromatography and gas chromatography was intended to improve the sensitivity achievable in the direct analysis of olive oils adulterated with virgin and refined hazelnut oils. The obtained results demonstrated the possibility of evaluating the genuineness of olive and hazelnut oils as well as of detecting adulterations of olive oil with percentages of around 5% and 10% of virgin and refined hazelnut oils, respectively, in less than 30 min by the method proposed.

Flores et al. (2006b) evaluated the presence or absence of filbertone in 21 admixtures of olive oil with virgin and refined hazelnut oil obtained using various processing techniques from different varieties and geographical origins by solid phase microextraction and multidimensional gas chromatography (SPME–MDGC). The obtained results showed that the sensitivity achievable with the proposed procedure was enough to detect filbertone and, hence, to establish the adulteration of olive oil of different varieties with virgin hazelnut oils in percentages of up to 7%. The very low concentrations in which filbertone occurs in some refined hazelnut oils made difficult its detection in specific admixtures. In any case, the minimum adulteration level to be detected depends on the oil varieties present in the adulterated samples.

Lagarda et al. (2006) indicated that phytosterols are bioactive compounds, one of their most studied and outstanding properties being their cholesterol-lowering activity. This explains the growing interest in the phytosterol contents of foods as either intrinsic or added components. According to their sensitivity and precision, the currently available techniques for sterol analysis can be classified as follows: GC > HPLC > SFC. The sensitivity order may vary depending on the sterol structures and detectors coupled to the chromatographic instruments. As reported, GC–FID (or MS, when peak identity confirmation is needed) can be considered the method of choice for the determination of phytosterols in foods and diets.

Cañabate-Díaz et al. (2007) investigated the first liquid chromatographic method for the identification and quantification of seven phytosterols in olive oil. Sterols were identified and quantified by liquid chromatography with mass spectrometry (LC-MS) detection in positive APCI (atmospheric pressure chemical ionization) mode. Sterol contents were determined in a virgin olive oil, a refined olive oil, an olive-pomace oil and a crude olive-pomace oil. A sufficient separation of cholesterol, stigmasterol, β-sitosterol, sitostanol, fucosterol, erythrodiol and uvaol was achieved and the method satisfactorily applied to different real samples.

Manai et al. (2008) evaluated the volatile composition of virgin olive oil (VOO) from six Tunisian new cultivars using headspace solid-phase microextraction method coupled to GC–MS and GC–FID. Various chemical classes were identified such as aldehydes, alcohols, esters, ketones, terpenes and carboxylic acids. The results showed that the most important contributors to olive oil aroma were C6 aldehydes. These compounds are biogenerated from polyunsaturated fatty acids through the lipoxygenase pathway. They pointed out the predominance of linolenic acid oxidation over linoleic acid one. The main volatile compounds present in the oil samples were C6 derivatives such as (E)-hex-2-enal, (E)-hex-2-enol and hexanol. In addition to C6 compounds, the aroma of the studied VOOs contains reasonable amounts of various classes of C5 components. The tested oil samples showed different volatile profiles. Some components seem to occur exclusively in a specific oil samples and, consequently, their presence might be initially used to establish compositional differences. All results put in evidence that olive oil aroma compounds accumulate differently according to the cultivar.

Mildner-Szkudlarz and Jeleń (2008) investigated the effectiveness of three rapid methods of volatile compounds analysis with subsequent principal component analysis (PCA) treatment of data for differentiation between virgin olive oil samples adulterated with hazelnut oil. Tested methods included comparison of chromatograms of volatiles obtained using SPME-fast GC FID, volatiles analysis by electronic nose based on MOS sensors (HS E-nose) and by direct coupling of SPME to MS (SPME-MS). Volatile compounds were analyzed also by SPME-GC/MS technique. Data obtained as a result of SPME-GC/MS was subjected to PCA. SPME-GC–MS analysis with subsequent PCA yielded good results, however being time consuming. The three methods of analysis of volatiles allowed detection of olive oil adulteration with different contents of hazelnut oil ranging from 5 to 50% (v/v).

Saitta et al. (2009) analyzed the phenolic fraction of 34 virgin olive oils by GC–MS to identify new compounds at low level. 27 compounds previously described in olive oils were identified; several new minor compounds with phenolic structure were detected in the samples: amongst them, 4-hydroxyphenylacetaldehyde, *trans*-isoeugenol (*trans*-2-methoxy-4-(1-propenyl)-phenol), 1,4-dihydroxy-2,6-dimethoxybenzene, 3,4-dihydroxybenzyl alcohol and 3,4-dihydroxyphenylacetic acid were identified by their mass spectra and confirmed using standards. In 34 virgin olive oils (cv. Nocellara del Belice) the mean concentrations for these five substances were always below 0.2 mg kg^{-1} and only in two samples the level of 3,4-dihydroxyphenylacetic acid reached 1.47 and 1.97 mg kg^{-1}, respectively. These compounds could be used to characterise olive oils; low concentrations of 3,4-dihydroxyphenylacetic acid may show the initial autoxidation processes of olive oils.

Vaz-Freire et al. (2009) performed the analysis of the aroma compounds after headspace-solid phase micro extraction. The analytical results obtained after comprehensive gas chromatography in tandem with time of flight mass spectrometry (GC×GC/ToFMS) for three different olive oil varieties, from a single year harvest and processed with two different extraction technologies and compared using statistical image treatment, by means of *ImageJ* software, for fingerprint recognitions and compared with PCA when the area data of each chromatographic spot of the contour plots were considered. The results obtained from this study showed that, when the right software is associated with the GC×GC chromatograms, it is easy to perform a quick and easy fingerprinting analysis, precluding the alignment of the contour plots obtained, which in our study allowed the identification of varieties as well as extraction technologies used to produce high quality olive oils. These results open the possibility of applying the methodology for authenticity and fraud control purposes, and also for quick matrix characterization, even when operated by non-experts, due to the simplicity of the methods involved.

2.6- Detection of Adulteration of Virgin Olive Oil by UV Spectroscopy

Kapoulas and Andrikopoulos (1987) utilized the ultra violet (UV) spectroscopic characteristics in the detection of adulteration of virgin olive oil with low proportions of refined olive, olive kernel, olive and seed oils. They found weak absorption maxima of conjugated tetraenoic systems (at 285 – 315 nm) that could be quantified by second-derivative spectrophotometry. Absorptivity (K_{315}) showed the differences between virgin olive oil, refined olive, olive kernel and seed oils. It was concluded that 5% adulteration of virgin olive oil by refined seed oils may be detected easily.

2.7- Detection of Adulteration of Virgin Olive Oil by IR Spectroscopy

Gauglitz and Vo-Dinh (2003) stated that infrared (IR) spectroscopy is a rapid, nondestructive and sensitive method easy to handle and provide all sampling techniques for gases, liquids and solids. Important aspects are the convenient qualitative and quantitative evaluation of the spectra. The mid- (fundamental) infrared region (IR or MIR) extends from 4000 cm^{-1} to 400 cm^{-1}. It is surrounded by the far-IR region (FIR) from 400 cm^{-1} to 10 cm^{-1} and the very important near-IR region (NIR) from 12500 cm^{-1} to 4000 cm^{-1}. The features of an IR spectrum (number of infrared absorption bands, their intensities and their shapes) are directly related to the molecular structure of a compound. The IR spectrum is a unique physical property of an individual compound; it is its molecular fingerprint. The IR region comprises fundamental vibrations of bound atoms. Whenever such bound atoms vibrate, they absorb infrared energy, i.e. they exhibit IR absorption bands. The condition for a normal vibration to be IR active is a change in molecular dipole moment during vibration.

With respect to the direction of the vibrational movement it may distinguish between stretching vibrations (changes of bond lengths) and deformation vibrations (changes of bond angles). Deformation vibrations may be subdivided into bending modes, twisting or torsion modes, wagging modes and rocking modes. Further subdivision refers to the symmetry of the vibration (e. g., symmetric or antisymmetric, in-plane or out-of-plane). Complications in evaluation of IR spectra are the overlapping of individual bands and the appearance of additional bands, e.g. overtone and combination bands, which may be caused by anharmonicity of some vibrations. In the NIR region, all bands are overtone or combination bands. They are always weaker in intensity than the corresponding fundamental bands. Originally considered as a drawback, the weak intensity of the NIR bands turned out to be the background for the large success of NIR spectroscopy in process analysis **(Gauglitz and Vo-Dinh, 2003).**

Detection of adulteration of virgin olive oil by IR spectroscopy was investigated by several investigators as following:

Wesley et al. (1995) carried out a method for predicting the level of adulteration in a set of virgin and extra virgin olive oils adulterated with corn oil, sunflower oil and raw olive residue oil by near-infrared spectroscopy. The best result was a correct prediction for 98% of the samples and 75% prediction rate. Principal component analysis was used to predict the type of adulterant. From these results it was concluded that it was possible to design a quality control system, which uses near-infrared technology to measure the level of adulteration. The final equation calculated from the calibration set had 14 factors and was checked by predicting the validation set and comparing results with the known laboratory values. There was obviously some interest in whether any of the factors used in the equations can be related to specific adulterant type. It was sometimes possible to identify such features by plotting the equation loadings. In this case, it was not possible to assign specific features to the different adulterants although interesting features were noted in the C–H overtone regions.

Lai et al. (1995) investigated the potential of IR spectroscopy for the quantitative determination of adulterants in extra virgin olive oil. They used FTIR combined with ATR and PLS regression for investigating two types of "contaminant" oil-refined olive and walnut. IR spectroscopy has been shown to be able to discriminate between different vegetable oils, and types of olive oil in particular. It was also possible to determine, quantitatively, the level of a typical adulterant in extra virgin olive oil. Although the lowest SEP (0-68 g/100 g) was observed when walnut oil was added to extra virgin olive oil, the technique clearly also has the potential to determine the addition of low levels of refined olive oil.

Wesley et al. (1996) presented the application of discriminant analysis for identifying and quantifying adulterants in extra virgin olive oils. Three adulterants were used (sunflower oil, rapeseed oil and soybean oil) and were present in the range of 5-95%. NIR spectroscopy and PCA were used to develop a discriminant analysis equation that could identify correctly the type of seed oil present in extra virgin olive oil in 90% of cases. PLS analysis was used to develop a calibration equation that could predict the level of adulteration. Cross validation suggested that it was possible to measure the level of adulteration to an accuracy of ±0.9%.

External validation of the derived calibration equation gave a standard error of performance of ±2.77%.

Baeten et al. (1996) utilized the combination of Fourier transform-Raman (FT-Raman) spectroscopy with multivariate procedures for predicting the level of adulteration in a set of virgin olive oil samples that were adulterated with soybean, corn, and raw olive residue (olive-pomace) oils at 1, 5, and 10% respectively. FT-Raman calibration was carried out with virgin olive oil of the Coratina variety. Six samples of genuine virgin olive oil adulterated with 99% trilinolein (LLL) in the range of 1-10% (w/w), plus an unadulterated control were used in this process. Six virgin olive oils, obtained from varieties Hojiblanca (3) and Picual (3) were the genuine samples selected. Each of theses samples was adulterated with soybean, corn, or olive pomace oils at 1, 5, or 10% (w/w), respectively. The LLL was present in virgin olive oil at trace levels; hence, the addition of LLL emulated the adulteration of virgin olive oil with other kinds of vegetable oils. The selected three oils were characterized by different levels of LLL. Verification of the wavenumbers selected in the calibration and the advantages of using supplementary wavenumbers in detecting adulteration was done. The results showed the ability of the Raman technique to detect the percentages of LLL in spiked virgin olive oil. The best result in prediction of adulteration was determined by PCR giving 100% correct discrimination between genuine and adulterated samples and 91.3% correct classifications at different adulteration levels.

Guillén and Cabo (1999) carried out a detailed investigation using FTIR spectroscopy to determine the usefulness of some FTIR spectroscopic bands for evaluating the composition of edible oils mixtures. Twelve sets of olive oil mixed with various edible seed oils in different proportions were made. The seed oils used were sunflower, corn, walnut, rapeseed, soybean, safflower, peanut, wheat germ, and sesame oil. These samples have very different proportions of saturated, mono- and polyunsaturated acyl groups, and of minor components. Fourier transformed infrared spectra of these blends were recorded from films of the oil samples between two discs of KBr. Taking into account the close relationships found previously between the frequency data of some specific bands and the composition of the oil samples, frequency data of all samples were collected and used in equations that relate frequency and composition predicting the percentage by weight of saturated, mono- and polyunsaturated acyl groups in the samples. The predicted values were compared with those derived from the combination of chemical and gas-chromatographic methods and a high degree of agreement was found. The presence of small amounts of seed oil in olive oil is shown by a small variation in the values of the frequencies of specific bands of the spectra, resulting from a smaller proportion of mono-unsaturated acyl groups than in pure olive oil. On the other hand, the frequency of the maximum absorbance between 915 cm^{-1} and 904 cm^{-1} also indicates the proportion of seed oil in the blend up to levels of 5% or up to 8% of seed oil in olive oil, depending on the nature of the seed oil. This methodology could give information about the composition of blends of edible oils in a very fast and simple way.

Yang and Irudayaraj (2001) carried out a comparison study between several spectroscopic techniques in the infra red (IR) region. Near infrared (NIR), mid infrared (MIR), Fourier transform-infrared (FTIR) and Fourier transform-Raman (FT-Raman) spectroscopic techniques were used to quantify the amount of olive-pomace oil adulteration in extra virgin olive oil. The concentration of olive-pomace oil in extra virgin olive oil was in the range between 0 and 100% in 5% increments by weight. The peaks in the NIR region (9091-4000 cm^{-1}) were broad and weak due to combinations and overtones of functional groups of sample chemical constituents, and hence NIR was mostly used in quantitative analysis. Unlike NIR, most of the peaks in the MIR region (4000-400 cm^{-1}) were narrow and sharp due to fundamental vibrations of molecules. Compared to dispersive NIR, FTIR spectroscopy in the MIR region had higher signal/noise ratio and resolution. FT-Raman spectroscopy can be regarded as a complementary method to the IR technique for analysis. Like FTIR spectroscopy, FT-Raman can also provide information on the functional/chemical groups for qualitative and quantitative characterization.

A comparison study of FTIR and FT-Raman techniques for the authentication of olive oil gave a classification accuracy of 100% using FTIR spectroscopy and 93.1% using FT-Raman spectroscopy. The adulteration detection limits were 5% for the adulterants of seed oils and refined olive oil for IR data, while they were 45% for refined olive oil and 5% for seed oils for Raman data. Comparison of the prediction models from the different spectroscopic methods indicated that FT-Raman spectroscopy gave the highest correlation (R^2 = 0.997) with the lowest prediction error (SEP = 1.72%). However, NIR and MIR techniques also provided good predictions with an R^2 values greater then 0.990. The ease of evaluation operation merits the use of IR and Raman techniques for adulterant determination studies. The techniques presented could also be used to study the adulteration of extra virgin olive oil with other lower-quality oils **(Yang and Irudayaraj, 2001).**

Tay et al. (2002) focused in their studies on the authentication of olive oil adulterated with vegetable oils using FT-IR Spectroscopy. Single-bounce attenuated total reflectance (ATR) measurements were made on pure olive oil and olive oil samples adulterated with varying concentrations of sunflower oil. Discriminant analysis using 12 principal components was able to classify the samples as pure and adulterated olive oils based on their spectra. A PLS model was developed and used to verify the concentrations of the adulterant. The typical spectra of extra virgin olive oil and sunflower oil were obtained. The prominent peaks due to C–H stretching mode in the wavenumber region of 2800–3100 cm^{-1}, C=O stretching in the region of 1700–1800 cm^{-1} and C–O–C stretching and C-H bending in the region of 900–1400 cm^{-1} can be easily observed. Most of the spectral information used for the discriminant analysis is contained in the wavenumber regions of 3100–2800 cm^{-1} and 1800–900 cm^{-1}. They observed that the presence of sunflower oil (as adulterant) in extra virgin olive oil can be detected through FT-IR spectroscopy with ATR and that the discriminant analysis can be used to classify pure vs. adulterated olive oil samples successfully down to an adulteration level of 20mL of sunflower oil in 1 L of extra virgin olive oil. This is much lower than the adulteration level at which such adulteration becomes economically significant.

Christy et al. (2004) developed a new procedure for the classification and quantification of the adulteration of pure olive oil by soya oil, sunflower oil, corn oil, walnut oil and hazelnut oil. The study was based on a chemometric analysis of the near-infrared (NIR) spectra of olive oil mixtures containing different adulterants with different concentrations. NIR spectra of the adulterated mixtures were measured in the region of 1200-4000 cm^{-1}. The spectra were subjected batchwise to multiplicative signal correction (MSC) before calculating the principal component (PCA) models. The MSC-corrected data were subjected to Savtzky-Galay smoothing and a mean normalization procedure before developing partial least squares calibration (PLS) models. The results showed the small differences in the models used for calibrations mostly come from the overtones and combinations of the $–CH_2–$ and $CH_3–$ stretchings in the oil glycerides. The compositions of the vegetable oils vary with the different fatty acids in the glyceride molecules. Different oils have different fatty acid composition, and there should be differences in the intensities of bands arising from the overtones and combinations of the $–CH_3$ and $–CH_2$ stretching vibrations. However, these differences appear to be smaller than expected, and the smaller percentage of the variances is responsible for explaining the variances in the adulterant concentrations. The calibrations gave excellent predictions for the percentages of adulterants when predicted by relevant models. The results revealed that the models predicted the adulterants; corn oil, sunflower oil, soya oil, walnut oil and hazelnut oil involved in olive oil within acceptable error limits being ± 0.57, ±1.32, ±0.96, ±0.56, and ±0.57% (weight/weight), respectively.

Yang et al. (2005) utilized FTIR, FT-NIR and FT-Raman spectroscopy for discrimination among 10 different edible oils and fats, and to compare the performance of these spectroscopic methods for edible oil/fat study. It is well known that every oil/fat differs in composition, length and unsaturated degree of the fatty acids as well as their positions in the chain. IR and Raman spectra represent a combined fingerprint pattern unique to each oil/fat and were used for discriminant analysis. A second set of analysis was conducted using regions specific to the C=C bond vibration. The double bond considered is an unsaturated bond and the intensities, areas or heights of its peak in this region might indicate the degree of unsaturation in fatty acids, sterols, and vitamins. The major peaks that represent triglyceride functional groups could be observed around 2937 cm^{-1} [C–H stretching (asymmetry)], 2856 cm^{-1} [C–H stretching (symmetry)], 1749 cm^{-1} [C=O stretching], 1454 cm^{-1} [C–H bending (scissoring)], 1166 cm^{-1} [C–O stretching and C–H bending], and 709 cm^{-1} [C–H bending (rocking)] There was a very weak peak around 1650 cm^{-1} in IR spectra, which is C=C stretching (*cis*).

The classifications of edible oils and fats under investigation were performed by applying discriminant analysis using FTIR spectra between 400 and 4000 cm^{-1}. The whole spectra between 400 and 4000 cm^{-1} are the combination of many constituents of oil/fat whereas the region selected between 1400 and 1800 cm^{-1} mostly represents the combination of C–H bending, C=O stretching, and C=C stretching and hence is directly related to unsaturated C=C bond. Generally, FTIR, FT-NIR and FT-Raman spectroscopy techniques can be used for rapid classifying edible oils and fats without the need for sample preparation. Both FTIR and FT-Raman spectroscopy techniques provided exquisite structural insights into functional groups of oils and fats for discriminant analysis. FTIR spectroscopy was found to be the most superior and efficient for discrimination and classification of oils and fats when used with CVA and yielded about 98% classification accuracy, followed by FT-Raman (94%) and FT-NIR (93%) methods **(Yang et al., 2005).**

Vlachos et al. (2006) used FT-IR spectroscopy as an effective analytical tool in order to determine extra virgin olive oil adulteration with lower priced vegetable oils (sunflower oil, soybean oil, sesame oil, corn oil) and to monitor the oxidation process of corn oil samples undergone during heating or/and exposure to ultraviolet radiation. A band shift observed at 3009 cm^{-1} assigned to the C–H stretching vibration of the *cis*- double bond (=CH) allows the determination of extra virgin olive oil adulteration. The oil composition affects the exact position of the band and yields shifts when the proportion of the fatty acid changes. In this way, the value of this frequency in non-oxidized oil samples varies significantly from 3009 to 3006 cm^{-1}. For example, sunflower oil, soybean oil, corn oil and sesame seed oil showed a maximum absorbance around 3009 cm^{-1} comparing to extra virgin olive oil that had a maximum of absorbance around 3006 cm^{-1}. This was due to their composition, as vegetable oils contain certain higher proportion of linolenic or linoleic acyl groups whereas extra virgin olive oil contains higher proportion of oleic acyl groups. Moreover, as the sunflower oil was gradually mixed in various proportions with extra virgin olive oil, the clear shift of the 3009 cm^{-1} band, attributed to the C–H stretching vibration of *cis*- double bond, to the 3005 cm^{-1} band was noticed. In order to quantify further the adulteration, the maximum heights of the two bands around 3006 and 2925 cm^{-1} were also used. The 2925 cm^{-1} band is attributed to the symmetric stretching vibration of the aliphatic CH_2 group. The height of the 3006 cm^{-1} band for the extra virgin olive oil is obviously smaller than it is for all the types of vegetable oils, and changes according to the extent of adulteration. When the percentage of added vegetable oil increased, the height of this band also increased, approaching the height that matches to the vegetable oil, when the adulteration was quite high. The 2925 cm^{-1} band height exhibits small changes and not in a specific way, as the 3006 cm^{-1} band. This study indicated that the detection limit for oil adulteration was 9% if the adulterant was corn oil or sesame seed oil while it was lower (6%) if the adulterant was sunflower oil or soybean oil.

Christy and Egeberg (2006) carried out quantitative determination of saturated and unsaturated fatty acids in edible oils by IR spectroscopy and chemometrics. Edible oils contain high proportion of unsaturated fatty acids. The proportion of the oleic to linoleic acids varies in the edible oils. For example, olive oil contains 85% of oleic acid compared to 39% in soya oil. At the same time soya oil contain 54% of linoleic acid compared to 9% in olive oil. The fatty acids of eight edible oils (coconut, olive, soya, cottonseed, sunflower, palm, walnut, and hazelnut oils) which have varying composition were saponified, esterified into methyl esters and analysed by GC. These composition values were used in calculating the composition of 25 synthetic edible oil mixtures containing a wide range of saturation and unsaturation. All these 33 oils were then analyzed by IR spectroscopy using single reflectance technique. The reflectance infrared profiles in 1/R format were calibrated against their saturated, unsaturated, 18:1 and 18:2 fatty acids composition using regions and the whole spectral profiles. Second derivated spectral profiles were also used in the calibration. The results showed that the cross-validated calibration model established with second derivated data of the infrared spectral profiles in the 4000–2700 cm^{-1} region against the GC evaluated unsaturated fatty acid composition predicted 5 unknown oil samples within an error limit of ±2.5%. The same was the case with the unsaturated fatty acid composition against second derivated infrared profiles in the region 4000–2700 cm^{-1}. Oleic acid and linoleic acid composition in the calibration oil samples were calibrated against the second derivated profiles of the IR spectra in the region 4000–600 cm^{-1}. The prediction values of the cross-validated calibration model for the unknown oil samples give oleic acid and linoleic acid composition in the oils with an error margin ±3.1% and ±4.1%, respectively. The calibration and prediction error includes the error in the quantitative determination of the fatty acids by GC. This method could be used to establish a quick screening for the determination of the quality of edible oils. The method saves a lot of time and money compared to the wet chemical methods.

Őzdemir and Őztűrk (2007) utilized FT-NIR spectroscopy to determine olive oil adulteration with sunflower and corn oil. They focused on the application of FT-NIR spectroscopy in conjunction with multivariate calibration to identify the adulteration of olive oils. FT-NIR transmittance measurements were made on pure olive oil and olive oil adulterated with varying concentrations (4-96%, v/v) of sunflower and corn oil in two sets of 26 binary and ternary mixtures. Multivariate calibration models were generated using genetic inverse least squares (GILS) method and used to predict the concentration of adulterants along with the concentration of olive oil in the samples. Over all, standard error of predictions ranged between 2.49 and 2.88% (v/v) for the binary mixtures of olive and sunflower oil and between 1.42 and 6.38% (v/v) for the ternary mixtures of olive, sunflower and corn oil.

Allam and Hamed (2007) carried out a study on the application of FTIR spectroscopic technique to identify the adulteration of extra virgin olive oil mixed with different concentrations of each of corn, sunflower and soybean oils (0, 25, 50, 75, or 100 %). The spectral region (1300-1000 cm^{-1}) which contains the IR fingerprints of these vegetable oils was found to be very useful in detecting olive oil adulteration. The intensities of the spectral bands at 1163 cm^{-1} (assigned to C–O stretching and CH_2– bending) increased, whereas the intensities of spectral bands at 1118 and 1097 cm^{-1} (assigned to C–O stretching) as well as the absorbance ratio (R1118/1097 cm^{-1}) of peak heights decreased with increasing adulterant concentration. Also, there was a pronounced shift of the peak at 912.78 cm^{-1} (assigned for HC=CH, *cis*-, bending out-of-plane) for pure olive oil to higher wave numbers with increasing adulterant concentration. Both search and quality control (QC) user libraries were created using Omnic Software and were used for spectral matching between pure olive oil and the adulterant oils. The accuracy of this method was validated by cross-referencing the actual and the predicted concentration of some samples containing known concentration of adulterant oils (forecasting samples). Results obtained by this technique were forecasting samples having accuracy up to 96.15 %.

Schulz and Baranska (2007) investigated the potential of IR and Raman spectroscopy for the authentication and detection of adulteration of vegetable oils. Especially IR methods are widely used as fast analytical techniques applied in agricultural, food and industrial laboratories. Advances in IR instrumentation (Fourier transform technique) and new sample presentation techniques such as total reflectance and photoacoustic detection have led to a widespread application of various IR methods in the area of vegetable oil analysis. Using discriminant analysis (DA) and principal component analysis (PCA) eight commercial vegetable oils (extra virgin olive oil, groundnut oil, corn oil, grape seed oil, olive oil, rape seed oil, sunflower oil, walnut oil) could be properly identified. When extra virgin and refined olive oils were subjected to a separate DA, all samples were correctly classified according to type. It has been found that the frequency of concrete absorption bands in the fingerprint region (700–1500 cm^{-1}) gives direct information about the ratio between saturated and *cis*- monounsaturated fatty acid acyl groups. Stretching vibrations of *trans*- and *cis*- olefinic double bonds have been observed around 3025 and 3006 cm^{-1}, respectively. Beside C–H stretching vibrations between 3000 and 2850 cm^{-1}, the C=O group of triglycerides shows strongest absorption bands at approx. 1746 cm^{-1}. A small shoulder to be seen at 1711 cm^{-1} is assigned to a small amount of free fatty acids. Several quantitative methods to characterize oxidation processes of vegetable oils have been also described in literature. A partial least squares (PLS) calibration model for the prediction of the peroxide value was developed based on spectral information in the range between 3750 and 3150 cm^{-1} which exhibits the characteristic hydroperoxide absorption bands, centered at 3444 cm^{-1}. The reproducibility of this FT-IR method was found to be better than the usually applied chemical titration method.

Sinelli et al. (2007) carried out a preliminary study on application of MIR spectroscopy for the evaluation of the virgin olive oil “Freshness”.

The freshness of virgin olive oils (VOO) from typical cultivars of Garda regions in Italy was evaluated by attenuated total reflectance (ATR) and Fourier transform infrared (FTIR) spectroscopy, in combination with multivariate analysis. The olive oil freshness decreased during storage mainly because of oxidation processes. 91 virgin olive oils were packaged in glass bottles and stored either in the light or in the dark at room temperature for different periods. The oils were analyzed, before and after storage, using both chemical methods and spectroscopic technique. Classification strategies investigated were partial least square discriminant analysis (PLS-DA), linear discriminant analysis (LDA), and soft independent modeling of class analogy (SIMCA). The results showed that ATR-MIR spectroscopy is an interesting technique compared with traditional chemical index in classifying olive oil samples stored in different conditions. The spectral data was subjected to analysis by SIMCA, developing models for fresh and oxidized VOOs (dark 1 year, dark 2 years and light 1 year). FTIR spectra of olive oils stored in different conditions were dominated by some peaks at 2924, 2852, 1743, 1463, 1377, 1238, 1163, 1114, 1099 and 721 cm^{-1}. Absorbance at 2924 and 2852 cm^{-1} were due to bands arising from CH_2 stretching vibrations, asymmetric and symmetric, respectively. The major peak at 1743 cm^{-1} arised from C=O stretching vibrations; the bands at 1463 and 1377 cm^{-1} arised from CH_2 and CH_3 scissoring vibration, while those at 1238, 1163, 1114, 1099 cm^{-1} were associated with the C–O stretching vibration. A small peak at 721 cm^{-1} corresponded to CH_2 rocking mode. In particular on PC1: 1743 cm^{-1}, corresponded to C=O stretching vibration of aldehydes and ketons; 1363 cm^{-1} corresponded to CH_3 scissoring vibration of ethers; 1218 cm^{-1} corresponded to C–O stretching vibration of epoxides. Variation in the mentioned absorption bands could be related to a higher concentration of aldehydes, ketons, epoxides in stored oil than in the fresh oils. FTIR results were compared with chemical data. FTIR PCA results allowed a better discrimination among fresh and oxidized oils, than samples separation obtained by PCA applied to chemical data.

Gurdeniz and Ozen (2009) carried out a study focusing on the detection and quantification of extra-virgin olive oil adulteration with different edible oils using mid-infrared (IR) spectroscopy with chemometrics. Mid-IR spectra were manipulated with wavelet compression previous to principal component analysis (PCA). The obtained IR spectra were dominated by peaks around 2800–3080 cm^{-1} which are due to hydrogen stretching mode. Large peak between 1700 and 1800 cm^{-1} could be attributed to C=O stretching, and C–O–C stretching and C–H bending in the region of 900–1400 cm^{-1} could be easily observed. Changes in the intensities of spectral bands of different oils are related to the composition of molecular bonds absorbing at those regions. There exists a notable difference between olive oil and adulterant oils in the peak between 3010 and 3000 cm^{-1} resulting from the C–H stretching vibration of the *cis*- double bonds (=CH). The intensity of this band changes slightly for each adulterant oil and olive oil significantly differs from them. The height of large peak around 2923 cm^{-1} is attributed to symmetrical stretching vibration of the aliphatic CH_2 group which varies between oils with higher absorbance values for olive oil. The availability of peak heights around 3006 cm^{-1} with the contribution of band around 2925 cm^{-1} to detect olive oil adulteration was illustrated. The band around 2853 cm^{-1} due to symmetrical stretching vibration of the aliphatic CH_2 group changes with respect to type of oil. There are also differences between the intensities of bands. A remarkable variation is observed in the peak heights around 1377 cm^{-1} resulting from bending vibrations of CH_2 groups. Adulterant oils exhibit some intensity in the band around 913–914 cm^{-1} whereas intensity and position of band is different for olive oil showing very low or no intensity. This important band was employed to detect and quantify adulteration.

Spectral regions which do not contain any relevant information were excluded in the further analysis and bands between 3120–2520 cm^{-1} and 1875.5–675 cm^{-1} were selected to be employed. The detection limit of adulteration was determined as 5% for corn–sunflower binary mixture, cottonseed and rapeseed oils. For quantification of adulteration, mid-IR spectral data were manipulated with orthogonal signal correction (OSC) and wavelet compression before partial least square (PLS) analysis. The results revealed that models predict the adulterants, corn–sunflower binary mixture, cottonseed and rapeseed oils, in olive oil with error limits of 1.04, 1.4 and 1.32, respectively. Furthermore, the data were analyzed with a general PCA model and PLS discriminant analysis (PLS-DA) to observe the efficiency of the model to detect adulteration regardless of the type of adulterant oil. In this case, detection limit for adulteration is determined as 10% **(Gurdeniz and Ozen, 2009).**

2.8- Detection of Adulteration of Virgin Olive Oil by NMR Spectroscopy

Guillén and Ruiz (2001) revealed that the high resolution ^{1}H NMR spectroscopy is a very useful technique in the study of several aspects of edible oils and fats. It has the advantages that it is not destructive, requires a very small sample whose preparation is very simple, and it takes little time. A single run detects all the protons present in the sample and provides signals whose intensity reflects the proportion of protons with the same local field, or in other words with the same chemical environment. The assignment of the signals to the protons of the different functional groups is, in general, well established; only some signals of some minor oil components have been tentatively assigned. So this technique enables the evaluation of both the major components of the sample and also of some minor components if their concentration is enough to be detected and if their signals do not overlap with those of the main components. These features make this technique very appropriate for the study of large numbers of samples in a very short period of time, providing a great deal of information. Its usefulness has been shown in the evaluation of important parameters for the food industry such as degree of unsaturation, proportions of the different acyl groups, authentication and quality assessment of oils, as well as oxidative oil stability. This technique provides a great deal of knowledge about oil and fat oxidation processes, which has great importance in studies of oil oxidation mechanisms and for the detection of derived products with potential toxicological properties, of interest for consumer health.

Gauglitz and Vo-Dinh (2003) reported that the magnetic resonance (NMR) spectroscopy is a flexible technique that gives detailed information for the selected nuclei, information about the chemical bonding, the local electronic structure, and the local site dynamics. NMR uses the very weak interaction between the nucleus and the rest of the universe. The interaction between the magnetic moment of the nucleus and the RF field of the NMR pulse/receiver circuit is extremely weak. However, the weak interaction also yields extremely high resolution and isolates the nucleus from the external perturbation for long periods; relaxation times of the order of seconds are common and, conversely, line widths can be less than 1 Hz. Small changes to the environment at the NMR-active nucleus can be detected and identified. For solutions, NMR observation of ^{1}H, ^{13}C, ^{15}N, and ^{31}P yields structures of organic molecules, organometallic complexes, proteins, and nucleic acid oligomers. For solids, ^{2}H and ^{13}C NMR yields polymer structure. For solids containing fluid inclusions, ^{1}H NMR yields porosity and diffusivity information, even from thousands of meters below ground with in situ NMR instruments lowered through boreholes into petroleum formations, airborne NMR was used to detect submarines, and satellite-mounted NMR mapped the Earth's magnetic field. Adding the magnetic field gradient yields an imaging experiment with magnetic resonance imaging (MRI): ^{1}H and ^{31}P MRI provide three-dimensional views of the human body, even showing specific brain activity. Even our breathing can be visualized with ^{129}Xe MRI.

In synthetic organic and organometallic chemistry, solution-state NMR means a 300-500 MHz NMR spectrometer, high-precision glass sample tubes, 2 ml of deuterated solvent (typically fully deuterated chloroform, acetone, benzene, or dichlorobenzene), several milligrams of the pure sample, and the basic suite of ^{1}H and ^{13}C NMR experiments **(Gauglitz and Vo-Dinh, 2003).**

Detection of adulteration of virgin olive oil by NMR spectroscopy was investigated by several investigators as following:

Mavromoustakos et al. (2000) found that the olefinic region of the ^{13}C nuclear magnetic resonance (^{13}C NMR) spectrum of virgin olive oil showed 12 peaks resonating between 127.5 and 130 ppm. These peaks were assigned to the most abundant unsaturated fatty acid moieties of the olive oil, oleic and linoleic acids, which are present in α and β positions of the glycerol backbone. These peaks correspond to the ethylenic carbons of the oleic (O) and linoleic (L) moieties present in α and β positions of the glycerol backbone, namely O-9α, O-9β, O-10α, O-10β, L-9α, L-9β, L-10α, L-10β, L-12α, L-12β, L-13α, L-13β. The area of each peak is expressed in mmol of fatty acid moiety per g of virgin olive oil using a known mass of pyrazine as an internal reference. The intensities of the 12 observed peaks were affected when an authentic virgin olive oil was mixed with a seed oil. To quantify the obtained NMR data, the area of a particular peak was directly compared to the area of the pyrazine peak which corresponds to a known mass content expressed in mmol. Pyrazine gives only a single peak at a chemical shift of 144.8 ppm. Thus, this peak is close to the region of interest and does not coincide with any of the observed peaks. This study focused on the olefinic region because it contains fewer overlapped peaks when compared to the carbonylic region of the spectrum. The major features observed in the series of spectra were: (i) As the relative concentration of soybean oil increased, a corresponding increase in the intensities of the linoleic acid peak and a decrease in the intensities of the oleic acid peak were observed. This was due to the fact that virgin olive oil contains an average of 73% oleic and 7% linoleic acids whereas soybean oil contains 23% oleic and 54% linoleic acids. (ii) Detection of adulteration through inspection of the linoleic acid content (mmol/g) is possible when the percentage of adulteration is $\geq$ 5%.

Vigli et al. (2003) used a combination of ^{1}H NMR and ^{31}P NMR spectroscopy and multivariate statistical analysis to classify 192 samples from 13 types of vegetable oils namely; hazelnut, sunflower, corn, soybean, sesame, walnut, rapeseed, almond, palm, groundnut, safflower, coconut and virgin olive oils from various regions of Greece. 1,2-diglycerides, 1,3-diglycerides, the ratio of 1,2-diglycerides to the total diglycerides, acidity, iodine value and fatty acid composition determined upon analysis of the respective ^{1}H NMR and ^{31}P NMR spectra were selected as variables to establish a classification/prediction model by employing discriminant analysis. This model, obtained from 128 samples resulted in a significant discrimination among the different classes of oils, whereas 100% of correct validated assignments for 64 samples were obtained. Different artificial mixtures of olive-hazelnut, olive-corn, olive-sunflower and olive-soybean oils were prepared and analyzed by ^{1}H NMR and ^{31}P NMR spectroscopy. Subsequent discriminant analysis of the data allowed detection of adulteration as low as 5% (w/w), provided that fresh virgin olive oil samples were used, as reflected by their high 1,2-diglycerides to total diglycerides ratio ($D \geq 0.90$). Compared with conventional methods, NMR spectroscopy offers a number of advantages, among which speed appears to be the most important. This feature makes it very useful for fast screening of large numbers of samples and for setting up a comprehensive data bank of authentic oils.

Vlahov (2006) utilized the ^{13}C nuclear magnetic resonance spectroscopy in a first attempt to differentiate olive oil samples by grades. High resolution ^{13}C NMR Distortionless Enhancement by Polarization Transfer (DEPT) spectra of 137 olive oil samples from the four grades, extra virgin olive oils, olive oils, olive pomace oils and lampante olive oils, were measured. The data relative to the resonance intensities (variables) of the unsaturated carbons of oleate (C-9 and C-10) and linoleate (L-9, L-10 and L-12) chains attached at the 1,3- and 2-positions of triacylglycerols were analyzed by linear discriminant analysis. The 1,3- and 2- carbons of the glycerol moiety of triacylglycerols along with the

C-2, C-16 and C-18 resonance intensities of saturated, oleate and linoleate chains were also analyzed by linear discriminant analysis. The three discriminanting functions, which were calculated by using a stepwise variable selection algorithm, classified in the true group by cross-validation procedure, respectively, 76.9, 70.0, 94.4 and 100% of the extra virgin, olive oil, olive pomace oil and lampante olive oil grades. These results confirmed that the ^{13}C NMR methodology, when applied to the measurement of spectra in correspondence of olive oil samples of different grades, produced rigorously quantitative profiles of the triacylglycerol fraction of oils in terms of the intensities of C-2, C-16 and C-18 resonances of the saturated, oleate and linoleate chains, and of the intensities of the double bond carbon resonances of the unsaturated chains.

2.9- Detection of Adulteration of Virgin Olive Oil by Mass Spectroscopy

Mass spectroscopy (MS) is an analytical method in which free gaseous ions are produced and subsequently subjected to magnetic and electric fields in a high vacuum for analysis of mass/charge (*m/c*) ratios. Although initially developed predominantly for physico-chemical investigations, MS has found broad application since the 1950s in the analysis of more complex organic and small biochemical molecules. Initial applications of MS to the study of biological processes date back to the 1940s with stable isotope ratio measurements. Complex mixtures of compounds that were either volatile or could be derivatised to enhance volatility could be analyzed by combined GC-MS. However, access of MS to applications in life sciences only became possible after solving one of the central problems, the generation and gas phase transfer of intact, structurally relevant ions of biomacromolecules. In recent years, dramatic analytical developments and advances in instrumentation have rendered MS central to many problems in modern biopolymer analysis. These advances make it possible to determine molecular masses of large biomacromolecules to isotopic accuracies this gives the possibility of identifying, e.g. minute yet functionally critical posttranslational modifications of proteins, supramolecular biopolymer interactions and biomolecular recognition processes **(Gauglitz and Vo-Dinh, 2003).**

Detection of adulteration of virgin olive oil by Mass spectroscopy was investigated by several investigators as following:

Lorenzo et al. (2002) investigated the direct coupling of a headspace sampler to a mass spectrometer for the detection of adulterants in olive oil. Samples of olive oils were mixed with different proportions of sunflower oil and olive-pomace oil, and patterns of the volatile compounds in the original and mixed samples were generated. Application of the linear discriminant analysis technique to the data from the signals was sufficient to differentiate the adulterated from the non-adulterated oils and to discriminate the type of adulteration. The results obtained revealed 100% success in classification and close to 100% in prediction. The main advantages of the proposed methodology are the speed of analysis (since no prior sample preparation steps are required), low cost, and the simplicity of the measuring process.

Peña et al. (2005) stated that the adulteration of olive oil with hazelnut oil is one of the most difficult to detect due to the similar composition of hazelnut and olive oils; both virgin olive oil and olive oil were subjected to that kind of adulteration. They developed an analytical method to detect adulteration of virgin olive oils and olive oils with hazelnut oil by means of its analysis by a headspace autosampler directly coupled to a mass spectrometer used as detector (ChemSensor). Four different crude hazelnut oils from Turkey were employed for the development of the method. Multivariate regression techniques (partial least squares and principal components analysis) were applied to generate adequate regression models. Good values were obtained in both techniques for the parameters employed (standard errors of prediction (SEP) and prediction residual error sum of squares (PRESS) to evaluate its goodness. By this method, they found that the minimum adulteration levels of 7 and 15% can be detected in the refined olive and virgin olive oils, respectively.

Gómez-Ariza et al. (2006) reported that the use of fast and reliable analytical procedures for olive oil authentication is a priority demand due to its wide consumption and healthy benefits. Olive oil adulteration with other cheaper vegetable oils is a common practice that has to be detected and controlled. Rapid screening methods based on high resolution tandem mass spectrometry constitute today the option of choice due to sample handling simplicity and the elimination of the chromatographic step. The selection of the ionization source is critical and the comparison of their reliability necessary. The possibilities of the direct infusion electrospray ionization (ESI) and the recently introduced atmospheric pressure photospray ionization source (APPI), coupled to quadrupole time-of-flight (QqTOF), have been critically studied and compared to control olive oil adulteration. These techniques are very rapid (approximately 1 min per sample) and have high discrimination power to elucidate key components in the edible oils studied (olive, hazelnut, sunflower and corn). Nevertheless, both sources are complementary, being APPI more sensitive for monoacyl- and diacylglycerol fragment ions and ESI for triacylglycerols. In addition, methods reproducibility's are very high, especially for APPI source. Mixtures of olive oil with the others vegetable oils can be easily discriminated which has been tested by using principal components analysis (PCA) with both ESI-MS and APPI-MS spectra. Analogously, linear discriminant analysis (LDA) confirms methods reproducibility and detection of other oils used as adulterants, in particular hazelnut oil, which is especially difficult given its chemical similarity with olive oil.

2.10- Detection of Adulteration of Virgin Olive Oil by CL Spectroscopy

Chemiluminescence (CL)-based detection has become in the last years quite a useful detecting tool in liquid chromatography (HPLC) due to its simplicity, low cost and high sensitivity and selectivity, and the development in instrumentation. Minimal instrumentation is required and no external light source is needed; thus, the optical system is quite simple.

As a consequence, a wide variety of analytical methods have been developed in clinical, pharmaceutical, environmental and food analysis. Chemiluminescence (CL) is defined as the production of electromagnetic radiation (ultraviolet, visible or infrared) observed when a chemical reaction yields an electronically excited intermediate or product, which either luminesces (direct CL) or donates its energy to another molecule responsible for the emission (indirect or sensitized CL). As advantages inherent to CL techniques, it is possible to remark the basic instrumentation required and the simplification of the optical system because no external light source is needed. CL is often described as a dark-field technique: the absence of strong background light level, such as found in absorptiometric techniques, reduces the background signal and leads to improved detection limits. During the last years, several books, chapters and reviews have been published about the CL detection, principles and analytical applications in the liquid phase, including developments in instrumentation, and the use of CL as detection mode in flow injection analysis (FIA), HPLC, and capillary electrophoresis (CE). High performance liquid chromatography (HPLC) coupled to CL detection represents an interface between the selectivity of a powerful separation method and the sensitivity of this detection technique. This combination provides a high efficiency in separation and low detection limits inherent to CL systems **(Gámiz-Gracia et al., 2009).**

Papadopoulous et al. (2002) observed weak chemiluminescence (CL) emission in commercial Greek extra virgin olive oils and in refined seed oils such as sunflower oils as well as in corn oils with potassium superoxide in the aprotic solvent dimethoxyethylene. On measuring the CL of mixtures of extra virgin olive oils with the cheaper refined seed oils, calibrations were produced which can be used for the determination of the adulteration of olive oils with seed oils down to 3%. Furthermore, depending on the kind of oils, “low” authenticity-CL-factors for olive oils (0.8–2.15μmol l-1 gallic acid) and “high” for seed oils (4.5–11.2μmol l-1 gallic acid) were calculated. They showed that the weak CL of extra virgin olive oils as well as of seed oils (sunflowers or corn oils) gave significant differences in CL intensities which can be used to discriminate extra virgin olive oils from seed oils (authenticity factors) and possibly for the determination of the adulteration of extra virgin olive oils with cheaper seed oils down to 3%.

2.11- Detection of Adulteration of Virgin Olive Oil by Fluorescence Spectroscopy

Guimet et al. (2004) reported that discrimination between virgin olive oils and pure olive oils is of primary importance for controlling adulterations. They showed the potential usefulness of two multiway methods; unfold principal component analysis (U-PCA) and parallel factor analysis (PARAFAC), for the exploratory analysis of the two types of oils. Both methods were applied to the excitation–emission fluorescence matrices (EEFM) of olive oils and then compared the results with the ones obtained by multivariate principal component analysis (PCA) based on a fluorescence spectrum recorded at only one excitation wavelength. For U-PCA and PARAFAC, the ranges studied were λex = 300–400 nm, λem = 400–695 nm and λex = 300–400 nm, λem = 400–600 nm. The first range contained chlorophylls, whose peak was much more intense than those of the rest of species. The second range did not contain the chlorophylls peak but only the fluorescence spectra of the remaining compounds (oxidation products and Vitamin E). The three-component PARAFAC model on the second range was found to be the most interpretable. With this model, it could distinguish well between the two groups of oils and find the underlying fluorescent spectra of three families of compounds.

Sikorska et al. (2005) demonstrated the capability of the fluorescence techniques for characterizing and differentiating vegetable oils. Fluorescence emission spectroscopy was successfully used to characterize and discriminate the edible oil samples. Fluorescence measurements are simple and can be used to classify different types of oils. Several distinct spectral ranges, such as those corresponding to tocopherols and chloprophylls, could be identified in the spectra and used as markers for differentiation of oils with a high degree of accuracy. It was proved that the synchronous fluorescence method gives a possibility of classifying different classes of edible oils using a single scan. It is quite possible that this approach can also be used for quantitative evaluation of fluorescent constituents after an appropriate calibration. Such quantitative assay of some fluorescent pigments may be of interest for analytical purposes, as their concentration gives useful indications about cultivars. This method, which analyzes a complex mixture without separation, is extremely useful from the practical point of view. Fluorescence provides high sensitivity, simplicity and selectivity and may serve as a complement to other spectroscopic techniques used in edible oil analysis.

Guimet et al. (2005) performed a rapid detection of olive–pomace oil (OPO) adulteration in extra virgin olive oils (EVOOs) from the protected denomination of origin (PDO) “Siurana” using excitation–emission fluorescence spectroscopy (EEFS) and three-way methods of analysis. In this study the potential of EEFS and the three-way methods of analysis detected OPO adulteration in PDO “Siurana” olive oils at low levels (5%) of adulterant oil. First, they applied unfold principal component analysis (unfold-PCA) and parallel factor analysis (PARAFAC) for exploratory analysis. Then, they used the Hotelling T2 and Q statistics as a fast screening method for detecting adulteration. They showed that discrimination between non-adulterated and adulterated samples can be improved using Fisher’s linear discriminant analysis (LDA) and discriminant multi-way partial least squares (N-PLS) regression, the latter giving a 100% of correct classification. Finally, they quantify the level of adulteration using N-PLS.

Poulli et al. (2007) carried out rapid synchronous fluorescence analysis for virgin olive oil adulteration assessment. They could differentiate virgin olive from olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils using total synchronous fluorescence (TSyF) spectra. TSyF spectra are acquired by varying the excitation wavelength in the region 250–720 nm and the wavelength interval (Δk) in the region from 20 to 120 nm. It was found that adulterants can be discriminated from virgin olive oil using a wavelength interval of 20 nm and excitation wavelength region 315–400, 315–392, 315–375, 315–365, 315–375 and 315–360 for olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils, respectively. 31 virgin olive oil mixtures with each potential adulterant were prepared at varying levels with emphasis at low concentrations. PLS regression model was used to quantify adulteration. This technique was useful for detection of olive-pomace, corn, sunflower, soybean, rapeseed, and walnut oil in virgin olive oil at levels of 2.6%, 3.8%, 4.3%, 4.2%, 3.6%, and 13.8% (w/w), respectively.

2.12- Detection of Adulteration of Virgin Olive Oil by XRF Spectroscopy

X-ray fluorescence (XRF) analysis is a powerful analytical tool for the spectrochemical determination of almost all the elements present in a sample. XRF radiation is induced when photons of sufficiently high energy, emitted from an X-ray source, impinge on a material. These primary X-rays undergo interaction processes with the analyte atoms. High-energy photons induce ionization of inner shell electrons by the photoelectric effect and thus electron vacancies in inner shells (K, L, M,-) are created. The prompt transition of outer shell electrons into these vacancies within some 100 fs can cause the emission of characteristic fluorescence radiation. The working principle of XRF analysis is the measurement of wavelength or energy and intensity of the characteristic photons emitted from the sample. This allows the identification of the elements present in the analyte and the determination of their mass or concentration. All the information for the analysis is stored in the measured spectrum, which is a line spectrum with all characteristic lines superimposed above a certain fluctuating background. Other interaction processes, mainly the elastic and inelastic scattering of the primary radiation on sample and substrate, induce the background. Measurement of the spectrum of the emitted characteristic fluorescence radiation is performed using wavelength-dispersive (WD) and energy-dispersive (ED) spectrometers **(Gauglitz and Vo-Dinh, 2003).**

Bortoleto et al. (2005) described an innovative technique based on X-ray scattering applied to classify complex organic matrices of different vegetable oils by taking a 1 ml aliquot of sample, depositing it on the thin MylarTM film of a sample cell and submitting it to irradiation for 100 s in a conventional EDXRF equipment. The resulting spectra are processed through Principal Component Analysis (PCA), a chemometric tool, and a very convenient classification is obtained as a function of the origin of the oil. This classification can be used to verify oil adulterations. The X-ray fluorescence spectrometer is a common bench equipment, with a Rh tube as source of the X-rays. Vegetable oils from corn, canola, soybean, sunflower and olive (extra virgin and others) were analyzed and classifications were obtained using information from the scattered radiation. For the corn, canola, soybean and sunflower oil analyses, the PC1×PC4 score plots show distinct groups for each oil. These differences related to the variable fatty acid contents in the samples. Considering the discrimination between extra virgin and other olive oils, the PC1×PC2 scores plot shows two groups of samples. The main difference between them can be attributed to the total absence of water in extra virgin olive oils, since this first extraction is performed at room temperature without any kind of reagent. The technique is very useful to rapidly classify vegetable oil samples at very low cost, with the possibility of performing the measurements in situ with a portable instrument. The combination of X-ray scattering and PCA offers a valuable method for the classification of vegetable oils. This method is based on a new way of measurement, using a common bench XRF spectrometer, and is simpler and more rapid when compared to conventional techniques used for this purpose, such as chromatography, mass spectrometry and others. Another advantage that can be indicated is that data acquisition occurs without the need for any reagents. Using this new analytical procedure, it was possible to discriminate extra virgin olive oils from other kinds of olive oil and also authentic extra-virgin olive oils from those adulterated with soybean oil.

2.13- Detection of Adulteration of Virgin Olive Oil by Electronic Nose

Electronic noses are instruments which mimic the sense of smell. These devices are typically array of sensors used to detect and distinguish odors precisely in complex samples at low cost. The sensor array consists of broadly tuned (non-specific) sensors that are treated with a variety of odor-sensitive biological or chemical materials. An odor stimulus generates a characteristic fingerprint (or smellprint) from the sensor array. Patterns or fingerprints from known odors are used to construct a database and train a pattern recognition system so that unknown odors can subsequently be classified and identified. Electronic nose instruments are composed of three elements, namely: (i) a sample handling system, (ii) a detection system, and (iii) a data processing system. These features make the electronic nose as a very useful tool for diverse applications in the food, cosmetic and pharmaceutical industry as well as in environmental control or clinical diagnostics **(Peris and Escuder-Gilabert, 2009).**

Guadarrama et al. (2001) reported the use of an electronic nose purposely designed for the organoleptic characterization of olive oil. The instrument works using an array of electrodeposited conducting polymer-based sensors. Such an array has been able to distinguish among not only olive oils of different qualities (extra virgin, virgin, ordinary and lampante) but also among Spanish olive oils prepared from different varieties of olives and even of different geographic origins. The performance characteristics of the electronic device have been tested by using a selection of well defined samples (different varieties of olive, geographic origins, organoleptic characteristics, etc). Such a selection has been carried out by experts in the field, in collaboration with olive oil producers. The results have been compared with those obtained by chemical analysis and with sensory analysis. The analyses were conducted with a purposely designed system combining an array of 16 conducting polymer gas sensors with a static headspace auto sampler unit. The complete device was monitored by a dedicated software including PCA. The response of the array of polymeric sensors, associated with multivariate analysis methods, facilitates the clustering and differentiation of the examined olive oils. The prototype of electronic nose using a sensor array based on conducting polymers purposely designed for the detection of olive oil aroma has been successfully used for the discrimination of very similar types of oils whose only difference was the geographic origin inside a small area.

Cosio et al. (2006) used an electronic nose and an electronic tongue, in combination with multivariate analysis, to verify the geographical origin and the uniqueness of specific extra virgin olive oils. The olive oil samples belong to a small production located in the lake of Garda (north of Italy) and distinguished with a European Protected Denomination of Origin trademark since 1998. In order to obtain a complete description of oil samples, free acidity, peroxide value, ultraviolet indices, and phenol content have been also determined. The dataset includes 36 Garda oils and 17 oils from other regions. Total phenols, important for organoleptic and antioxidant characteristics, have been evaluated to verify a possible correlation with the geographical origin of oil samples. Two classification models have been built by means of Counterpropagation Artificial Neural Networks in order to separate Garda and not-Garda oils, as follows: first, by using all the chemical variables and sensor signals; second, by using electronic tongue sensors; finally, by using four selected electronic nose sensors. All the models have been also tested with 19 commercial olive oil samples. Neural networks have provided very satisfactory results and have indicated the electronic nose as the most appropriate tool for the characterization of the analyzed oils. These results have suggested how electronic nose, in combination with neural networks, could represent a fast, cheap and functional method to classify and describe extra virgin olive oils from a circumscribed geographical area.

3- MATERIALS AND METHODS

3.1- MATERIALS

The main raw materials which used for preparation of admixtures under investigation were as following:

3.1.1- Oils

3.1.1.1- Extra virgin olive oil (EVOO): The ripe and green olive fruits (Kronaky cultivar variety) were brought during seasons of 2005 from Horticulture Research Institute Farm. The olive oil was extracted from fresh olive fruits by Oliomio-machine with capacity of 30 Kg olive fruits/hour in Olive Research Department/Horticulture Research Institute. The Oliomio-machine consists of metal crusher unit, malaxation unit, and two-phases centrifugal decanter which are all working to separate the olive paste into vegetable water and pomace together from an one outlet and olive oil from the other outlet, then filtered through a filter paper and kept in brown glass bottles at -5°C till analysis.

3.1.1.2- Refined olive oil (ROO): Refined olive oil of Italian origin was bought from supermarket in the Kingdom of Saudi Arabia (KSA).

3.1.1.3- Sunflower oil (SO): Refined, bleached and deodorized (RBD) sunflower oil was obtained from Arma Company for Edible Oils, Egypt.

3.1.1.4- Corn oil (CO): Refined, bleached and deodorized (RBD) corn oil was obtained from Arma Company for Edible Oils, Egypt.

3.1.2- Solvents:

Hexane, petroleum ether, diethyl ether, chloroform, acetone, ethanol, and methanol (Analytical grade) were obtained from Al-Gomhoria Company for Drugs Trading and Chemicals, Egypt.

3.1.3- Standards:

3.1.3.1- Standard methyl esters of palmitic, palmitoleic, heptadecanoic, heptadecenoic, stearic, oleic, linoleic, linolenic, arachidic, eicosanoic, behenic and lignoceric acids were obtained from Sigma Chemical Company, USA.

3.2- METHODS

3.2.1- Preparation of Binary Admixtures Containing Different Adulterant Oils; (w/w %):

3.2.1.1- Binary admixtures of sunflower oil in extra virgin olive oil:

a- Pure olive oil (100% extra virgin olive oil)
b- Pure sunflower oil (100%)
c- 50% olive oil + 50% sunflower oil
d- 60% olive oil + 40% sunflower oil
e- 70% olive oil + 30% sunflower oil
f- 80% olive oil + 20% sunflower oil
g- 90% olive oil + 10% sunflower oil
h- 95% olive oil + 5% sunflower oil

3.2.1.2- Binary admixtures of corn oil in extra virgin olive oil:

a- Pure olive oil (100% extra virgin olive oil)
b- Pure corn oil (100%)
c- 50% olive oil + 50% corn oil
d- 60% olive oil + 40% corn oil
e- 70% olive oil + 30% corn oil
f- 80% olive oil + 20% corn oil
g- 90% olive oil + 10% corn oil
h- 95% olive oil + 5% corn oil

3.2.1.3- Binary admixtures of refined olive oil in extra virgin olive oil:

a- Pure olive oil (100% extra virgin olive oil)
b- Pure refined olive oil (100%)
c- 50% olive oil + 50% refined olive oil
d- 60% olive oil + 40% refined olive oil
e- 70% olive oil + 30% refined olive oil
f- 80% olive oil + 20% refined olive oil
g- 90% olive oil + 10% refined olive oil
h- 95% olive oil + 5% refined olive oil

3.2.2- ANALYTICAL METHODS

3.2.2.1- Refractive Index (RI):

The refractive index of oils was determined according to the method described in **AOAC (2000)**. Carl Zeiss refractometer was used, and the results were standardized at 25°C, applying the following relationship:

$R = R^{o} + K (T^{o} - T)$ where;
R = reading reduced to standard temperature,
R^{o} = reading obtained at temperature T^{o}
T = standard temperature
K = 0.000385

3.2.2.2- Acid Value (AV) and Free Acidity:

The free acidity and acid value of all samples were determined according to the method outlined in **AOAC (2000)**.

3.2.2.3- Peroxide Value (PV):

The peroxide value of oils was determined according to the procedure described in **AOAC (2000)**.

3.2.2.4- Iodine Value (IV):

The iodine value was determined according to Hanus method outlined in **AOAC (2000)**.

3.2.3- Ultraviolet (UV) Spectroscopic Characteristics:

Natural conjugated constituents are determined by measuring UV absorption at specific wavelengths in purified solvent.

The ultraviolet spectra of organic compounds are attributable to electronic excitations or transitions. Functional groups with high electron densities such as carbonyl groups, nitro groups, double, triple bonds and conjugated double bonds are usually considered. The sample was dissolved in freshly distilled (95%) ethanol to a known concentration. The absorption was then read at 232 nm for conjugated diene, and at 270 nm for conjugated triene in quartz cell with 1 cm path length against a solvent blank in a matching cell using a PyeUnicam double-beam spectrophotometer, model SP1800, as described by **Kates (1972)**, Absorptivity for conjugated constituents was calculated, applying the following relationship:

$$K = A / b\, c$$

where; A = observed absorbance

b = cell length in cm

c = g test portion / L final dilution used for K measurement

The ΔK value was calculated according to **IOOC (2001)** as following:

$$\Delta K = K_{268} - [\, (K_{262} + K_{274}) / 2 \,]$$

3.2.4- Gas Chromatography (GC) Analysis

3.2.4.1- Gas Chromatography Analysis of Fatty Acids:

The fatty acid composition was released as methyl ester according to **IOOC (2001)** as following:

In a 5 ml screw-top test tube weighed approximately 0.1 g of the oil sample. Then 2 ml of heptane was added and shaked well. Then 0.2 ml of 2N methanolic potassium hydroxide solution was added, put on the cap fitted with PTFE-Joint, the cap was then tightened and the tube was shaked vigourously for 30 seconds. Left to stratify until the upper solution became clear. Then the upper layer was decanted that contained the methyl esters. The heptane solution was then injected into the gas-liquid chromatography instrument (HP 6890N, Agilent Technologies, USA) equipped with a split/splitless injector (split ratio 1:100) with flame ionization detector (FID) and DB-23 capillary column of 60 m × 0.32 mm ID, 0.25 μm film thickness was filled with 50% cyano-propyl-methyl-poly-siloxane. The injector temperature was 250°C and the detector temperature was 275°C. The temperature of the column was programmed initially 150°C then allowed to rise at a rate of 10°C/min till reaches

170°C then allowed to rise at a rate of 5°/min till reaches 192°C then holding at this temperature for 4 min. then allowed to rise at a rate of 10°C/min till reaches 220°C then holding at this temperature for 3 min. Nitrogen gas was used as carrier gas with constant flow rate 3.0 ml/min. Hydrogen gas was used as fuel gas with constant rate 3.3 ml/min. and air with constant rate 33 ml/min. The volume injected was 1µl and injected into the column by a Hamilton micro syringe.

The fatty acids present in the sample under investigation were thus identified by comparison of relative retention times (RT) and peak areas which are estimated by computerized process, then these peak areas were used to calculate the fatty acid content expressed as percentage of total fatty acids, applying the following relationship:

Area % Fatty Acid$_X$ = $[A_X / A_T] \times 100$, where;
A_X = area counts of fatty acid methyl esters X
A_T = total area counts for chromatogram

The standard solution composed of standard methyl esters of fatty acids used for identification and matching of samples contained the following methyl esters of fatty acids: palmitic (16:0), palmitoleic (16:1), heptadecanoic (17:0), heptadecenoic (17:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), eicosanoic (20:1), behenic (22:0) and lignoceric (24:0) and this standard was obtained from Sigma Chemical Company, USA.

3.2.4.2- Useful Relationships Obtained from Fatty Acid Composition:

3.2.4.2.1- Oleic acid / Linoleic acid (C18:1/C18:2).
3.2.4.2.2- Palmitic acid / Linoleic acid (C16:0/C18:2).
3.2.4.2.2- Palmitic acid / Palmitoleic acid (C16:0/C16:1).
3.2.4.2.3- Saturated fatty acids / Unsaturated fatty acids (ΣSFA/ΣUSFA).

3.2.4.3- Determination of Total Unsaponifiable Matter:

The total unsaponifiable matter was determined according to the method outlined in **AOAC (2000).**

3.2.5- FTIR Spectroscopic Characteristics:

3.2.5.1- FTIR Spectral Data Analysis:

FTIR spectral analysis was carried out according to the method outlined by **Guillén and Cabo (1999)** as following:

A number of 18 sets of extra virgin olive oil mixed with each of the adulterant oils; sunflower, corn and refined olive oils in different proportions (5, 10, 20, 30, 40 and 50%) on w/w basis without sample preparation procedures, plus 4 sets of pure oils; extra virgin olive, sunflower, corn and refined olive oils, were exposed to FTIR analysis using a Jasco Spectrum FTIR Plus 460 Spectrophotometer (Japan) equipped with a deuterated tri-glycine sulphate (DTGS) detector was used to obtain FTIR spectra. The standard detector in routine FTIR instruments is the pyroelectric DTGS (deuterated tri-glycine sulfate) detector, whose response in the MIR range is wavelength independent. The MIR spectral range extends from 4000 to 400 cm^{-1}. The detector operates at ambient temperature and shows good linearity across the whole transmittance scale. The DTGS detector responds to signal frequencies of up to several thousand Hz, hence the time needed to scan one spectrum at a resolution of 4 cm^{-1} is of the order of 1 s. A small quantity (one drop of the sample) was pressed between two well-polished KBr disks (liquid cell) creating a thin film.

Each sample undergoes 64 scans which are accumulated in one measurement to acquire a sufficient signal-to-noise ratio. Samples were scanned between 4000 and 400 cm^{-1} with a nominal resolution of 4 cm^{-1} and the data interval was 1cm^{-1}. Time required for acquiring a complete sample spectrum was 30 second.

Fourier transformed infrared spectra of these blends were recorded taking into account the close relationships found previously between the frequency data of some specific bands and the composition of the oil samples, frequency data of all samples were collected and used in equations that relate frequency and composition.

3.2.5.2- Useful Relationships Obtained from FTIR Spectral Data Analysis:

3.2.5.2.1- Absorbance ratio values (R 1118/1097 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower, corn, and refined olive oils) at various concentrations were calculated and exposed to differential comparison illustrations.

3.2.5.2.2- Absorbance ratio values (R 1747/2925 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower, corn, and refined olive oils) at various concentrations were calculated and exposed to differential comparison illustrations.

3.2.5.2.3- Absorption intensity values (at 1163.83 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower, corn, and refined olive oils) at various concentrations were calculated and exposed to differential comparison illustrations.

3.2.5.2.4- Shift of absorption peaks (at 912.78 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower, corn, and refined olive oils) at various concentrations were calculated and exposed to differential comparison illustrations.

3.2.5.2.5- Shift of absorption peaks (at 3005.52 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower, corn, and refined olive oils) at various concentrations were calculated and exposed to differential comparison illustrations.

4- RESULTS AND DISCUSSION

4.1- Physical and Chemical Properties:

Some physical and chemical properties as refractive index, acid value, free acidity, peroxide value and iodine value of extra virgin olive oil alone and of its admixtures with different adulterant oils such as sunflower, corn, and refined olive oils at various concentrations were determined and the results are summarized in Tables (1, 2, 3, 4 and 5).

4.1.1- Refractive Index:

Refractive index (RI) is an important characteristic of oils because of the ease and speed with which it can be determined precisely, the small quantity of sample needed, and its relationship to structure. It generally gives a good idea about the degree of unsaturation of fats and oil, as well as its correlation with the iodine value.

The refractive indices of extra virgin olive, sunflower, corn, and refined olive oils and their admixtures were determined and the results are tabulated in Table (1).

From this table, it could be seen that the refractive index value measured at 20°C of extra virgin olive oil was the smallest one (1.4678) comparing to the values of 1.4722, 1.4755, and 1.4705 for sunflower oil, corn oil and refined olive oil, respectively. These variations in refractive indices of the corresponding oils under investigation were attributed to the differences in fatty acid composition of these oils containing different profiles, especially linoleic acid (C18:2) percentages. Extra virgin olive oil characterized by less linoleic acid percentage compared to sunflower and corn oils which contained substantially higher percentages of C18:2. These observations are coincided by **Ismael (1989); Mohamed (1999); Ahmed (2004); Abd El-Fattah (2007)** and also are supported by data obtained in this study (Tables 11, 12 and 13) which revealed that extra virgin olive oil contained C18:2 in a percentage of 6.21%; this was the least percentage corresponding to its least refractive index value of 1.4678

Table (1) Refractive index values of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations, at 20^{o}C

Adulterant oil	Refractive index values of binary admixtures at 20^{o}C		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	1.4678 (EVOO)	1.4678 (EVOO)	1.4678 (EVOO)
5	1.4685	1.4700	1.4680
10	1.4689	1.4704	1.4690
20	1.4695	1.4709	1.4698
30	1.4706	1.4716	1.4701
40	1.4708	1.4720	1.4702
50	1.4709	1.4722	1.4703
100	1.4722 (SO)	1.4755 (CO)	1.4705 (ROO)

EVOO; extra virgin olive oil
SO; sunflower oil
CO; corn oil
ROO; refined olive oil

compared with the other 3 oils under investigation which had percentages of C18:2 as 59.28%, 54.12% and 16.22% corresponding to sunflower, corn and refined olive oils, respectively. It is obviously clear that there is a direct proportional relationship between percentages of C18:2 and refractive indices. The more the increase in refractive index, the highly the degree of unsaturation, especially dienoic fatty acid in the oil.

The obtained refractive indices of extra virgin olive oil and refined olive oil are agreed with the limits for edible olive oil as reported in **Codex Alimentarius Standard (2003); Egyptian Standards (2005a)**, in which virgin olive oils show refractive index at 20°C ranged between 1.4677–1.4705, this range is the same for refined olive oil.

Also, the obtained results agreed with those found by **El-Qadi (1976); Khalil et al. (1983)** for pressed or solvent extracted olive oils; **El-Sharkawy et al. (1984)** who determined the refractive indices of three olive oil varieties; Shemlali, Mission and Frantio, the values obtained were as 1.4686, 1.4684–1.4688 and 1.4687–1.4696, respectively. **Mohamed (1999)** found that the refractive indices measured at 25°C for olive oils from different areas ranged between 1.4672–1.4675.

The refractive index of sunflower oil in this study agree with those reported by **Codex Alimentarius Standard (1999); Egyptian Standards (2005b); National Sunflower Association (2009)**, and also by other investigators; where their obtained values ranged between 1.4639-1.4746 at 25°C as evaluated by **Mohamed (1999); Ahmed (2004); Abd El-Fattah (2007); Manral et al. (2008)**.

The obtained refractive index of corn oil agree with the limits reported by **Codex Alimentarius Standard (1999); Corn Refiners Association (2006).** The measured value was also in accordance to those obtained by **El-Nikeety (1981); Ismael (1989)**, where its value was found to range between 1.4650-1.4770.

From Table (1) it could be seen that the addition of different concentrations from sunflower oil (5, 10, 20, 30, 40, and 50%) to extra virgin olive oil showed gradual increases in the refractive indices of these blends. The same observation was noticed in the case of addition of the same ratios either from corn oil or refined olive oil added to extra virgin olive oil since they have a higher refractive index; being 1.4722, 1.4755 and 1.4705 for sunflower, corn and refined olive oils, respectively, than that of extra virgin olive oil (1.4678).

The addition of 30% from sunflower oil caused the blend out of **Codex Alimentarius Standard (2003)** in which the refractive index of extra virgin olive oil and refined olive oil ranged from 1.4677 to 1.4705. This means that the adulteration of extra virgin olive oil with sunflower oil can be detected at 30% and above concentrations of sunflower oil by measuring the refractive index. In other words; the adulteration of extra virgin olive oil with sunflower oil can not be detected at concentration below 30% using the refractive index parameter.

While a binary admixture containing 20% corn oil caused the blend out of **Codex Alimentarius Standard (2003).** It means that the adulterant corn oil in binary admixtures with extra virgin olive oil can be detected when its concentration reached 20% and above by measuring the refractive index of the blend. In other words; the adulteration of extra virgin olive oil with corn oil can not be detected below 20% of its concentration using the refractive index as a monitor.

Concerning the adulteration with refined olive oil, the obtained refractive index data of all the adulterant concentrations in the binary admixtures were in the range of **Codex Alimentarius Standard (2003)** of extra virgin olive oil which ranged from 1.4677 to 1.4705, for virgin olive oil and refined olive oil. This means that the adulteration of extra virgin olive oil with refined olive oil can not be detected at any concentration, once it has the same established limits.

In conclusion, adulteration of extra virgin olive oil can be detected using refractive index as a monitor by the addition of 30% upward higher concentrations of the adulterant sunflower oil, and by the addition of 20% upward higher concentrations of the adulterant corn oil. Whereas, the adulterant refined olive oil can not be detected at any concentrations.

4.1.2- Acid Value and Free Acidity:

Acid value is a measure of the amount of free fatty acids present in the oil due to hydrolysis of its triglycerides.

The acid values of extra virgin olive oil and its admixtures with different concentrations of sunflower, corn and refined olive oils were determined and expressed as mg KOH/g oil and the results are presented in Table (2). It was also expressed as free fatty acids percentage as oleic acid and the results are presented in Table (3).

From Tables (2 and 3), it can be seen that the acid values and free fatty acids of extra virgin olive, sunflower, corn and refined olive oils were: 0.555 and 0.276%, 0.056 and 0.028%, 0.144 and 0.072%, 0.115 and 0.057%, respectively. It is also noticed that all oils used in adulteration had a lesser acidity than that of extra virgin olive oil. Therefore, addition of different concentrations of 5, 10, 20, 30, 40, and 50% from these oils to extra virgin olive oil caused pronounced decreases in the acidity of the blends produced.

The acid value or the free acidity have been frequently used as important parameters to monitor the quality of oils and to show the case of hydrolysis which is induced in the oil. The lesser values of acidity indicate that there was no hydrolysis produced in these oils beside that the refining process which was conducted for the vegetable oils used in this investigation.

These results agree with those reported by **Codex Alimentarius Standard (2003); Egyptian Standards (2005a); IOOC (2008)**, in which the free acidity of extra virgin olive oil ≤ 0.8 and refined olive oil ≤ 0.3. Also, the present data are in agreement with those of **El-Sharkawy et al. (1984)**. They found that the acidity of three olive oil varieties were; 0.350–0.810 for Shemlali, 0.290–0.570 for Mission and 0.0433–0.0498 for Frantio. While **Khalil (1987); Awatif et al. (1996); Mohamed (1999)** reported acidity ranged between 0.06-0.30 for virgin olive oil. Whereas, **Salvador et al. (2003)** found acidity value of Cornicabra virgin olive oil as 0.58. Also, **Torres and Maestri (2006)** reported free acidity values of

Table (2) Acid values of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil	Acid values of binary admixtures		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	0.550 (EVOO)	0.550 (EVOO)	0.550 (EVOO)
5	0.536	0.397	0.360
10	0.505	0.297	0.350
20	0.447	0.260	0.330
30	0.436	0.248	0.300
40	0.369	0.205	0.275
50	0.359	0.147	0.184
100	0.056 (SO)	0.144 (CO)	0.115 (ROO)

EVOO; extra virgin olive oil

SO; sunflower oil

CO; corn oil

ROO; refined olive oil

Table (3) Free acidity values of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil %	Free acidity values of binary admixtures		
	EVOO + SO	EVOO + CO	EVOO + ROO
0	0.276 (EVOO)	0.276 (EVOO)	0.276 (EVOO)
5	0.269	0.199	0.181
10	0.254	0.149	0.176
20	0.224	0.130	0.165
30	0.219	0.124	0.150
40	0.185	0.103	0.138
50	0.180	0.074	0.092
100	0.028 (SO)	0.072 (CO)	0.057 (ROO)

EVOO; extra virgin olive oil
SO; sunflower oil
CO; corn oil
ROO; refined olive oil

virgin olive oils from 4 olive varieties (Arbequina, Ascolana, Manzanilla and Nevadillo) cultivated at Traslasierra Valley, Córdoba, Argentina, which were exposed to the study of the influence of 2 extraction methods; two-phase centrifugation and pressure. The 4 varieties had the following free acidity values for the two-phase centrifugation method of extraction: 0.14, 0.40, 0.15 and 0.12, respectively. Whereas, they had the following free acidity values for the pressure method of extraction: 0.29, 1.03, 0.35, and 0.32, for the mentioned varieties, respectively. Also, **Haddada et al. (2007)** reported the free acidity values for 7 Tunisian virgin olive oils obtained from homogeneous olive fruits of 7 Tunisian olive cultivars as following; Chétoui 1: 0.30, Neb Jmel: 0.10, Regregui: 0.25, Rekhami: 0.20, Ain Jarboua: 0.25, Jarboui 1: 0.15 and Bidh Hmam: 0.25. Other investigators; **Pardo et al. (2007); Gómez-Alonso et al. (2007); Clodoveo et al. (2007); Bešter et al. (2008); Manai et al. (2008); Oueslati et al. (2009)** reported the free acidity values of extra virgin olive oil as ranged between 0.09-0.56. In this respect, **Espínola et al. (2009)** reported the acidity (%) of virgin olive oil which was obtained from 6 varieties; Picual 1, Picual 2, Picual 3, Picual 4, Hojiblanca and Arbequina, being as follows: 0.14, 0.16, 0.17, 0.24, 0.37 and 0.26, respectively. Also, the obtained acidity values of virgin olive oils agreed with those reported by **Baccouri et al. (2008); Runcio et al. (2008); Boselli et al. (2009).**

Among the obtained acid values of sunflower seed oil in this study, its value fell in range between: 0.05–0.25 as reported by **Ahmed (2004); Armenta et al. (2007); National Sunflower Association (2009).** Whereas, **Mohamed (1999); Abd El-Fattah (2007)** reported acid values of sunflower oil as 0.23 and 0.15, respectively. Also, the acid value of sunflower oil is ≤ 0.6 mg KOH/g oil and the acidity is ≤ 0.4% as reported by **Codex Alimentarius Standard (1999); Egyptian Standard (2005b).** However, the value obtained of sunflower was nearby to that evaluated (0.05%) by **Manral et al. (2008).**

The acid value of corn oil in this study was close to that determined by **El-Nikeety (1981)** as ranged between 0.04–0.40. Whereas, the acid value as reported by **Codex Alimentarius Standard (1999); Corn Refiners Association (2006)** is ≤ 0.6 mg KOH/g oil**.**

As shown in Tables (2 and 3), the addition of different concentrations from sunflower oil (5, 10, 20, 30, 40, and 50%) to extra virgin olive oil showed gradual decreases in the acid values and free acidity of these blends starting from pure extra virgin olive oil which showed an acid value and free acidity of 0.550 and 0.276%, respectively, till pure sunflower oil which showed acid value and free acidity of 0.056 and 0.028%, respectively.

The same observation was noticed in the case of the addition of the same ratios (5, 10, 20, 30, 40, and 50%) from the adulterant corn oil added to extra virgin olive oil, where these blends starting from pure extra virgin olive oil till pure corn oil showed gradual decreases in the acid values and free acidity values, since pure corn oil has a lower acid value and free acidity value; being 0.144 and 0.072%, respectively, than those of extra virgin olive oil (0.550 and 0.276%).

Similarly, the same observation was noticed in the case of addition of the same ratios (5, 10, 20, 30, 40, and 50%) from refined olive oil added to extra virgin olive oil, where these blends starting from pure extra virgin olive oil till pure refined olive oil showed gradual decreases in the acid values and free acidity values, since the pure refined olive oil has a lower acid value and free acidity values; being; 0.115 and 0.057, respectively, than those of extra virgin olive oil (0.550 and 0.276%).

In conclusion, it is clear that although the addition of different concentrations from each of sunflower, corn and refined olive oils (5, 10, 20, 30, 40, and 50%) to extra virgin olive oil showed gradual decreases in the acid values of these blends starting from extra virgin olive oil till pure sunflower, corn and refined olive oils, but these decrements were still present inside the range of acid value of extra virgin olive oil as reported by **Codex Alimentarius Standard (2003); IOOC (2008).** This means that adulteration of extra virgin olive oil with these adulterant oils can not be detected at any concentration by determining acid value and acidity.

4.1.3- Peroxide Value:

The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides. Therefore, it seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation. However, this theory is limited due to the transitory nature of the peroxides which are intermediate products in the formation route of carbonyl and hydroxyl compounds. Thus, the peroxide value indicates the case of oxidative rancidity of any oil and expressed as milliequivalent or mmole of peroxide per one kilogram of the given oil.

The peroxide values of extra virgin olive oil and its binary admixtures with adulterant sunflower, corn and refined olive oils at different concentrations of 0, 5, 10, 20, 30, 40, 50 and 100% were determined and the results are presented in Table (4). From this table, it could be observed that although peroxide value of extra virgin olive oil was a higher value being 10.80 m.equiv. than oils used in adulteration, it still agrees with that reported in **Codex Alimentarius Standard (2003); IOOC (2008)** for olive oils (extra virgin grade) which indicates that the peroxide value of olive oils does not exceed 20 m.equiv.

These obtained results of peroxide value of extra virgin olive oil agree with those reported by **Codex Alimentarius Standard (2003); Egyptian Standards (2005a); IOOC (2008)** where the peroxide value for virgin olive oil ≤ 20 of milliequivalents of active oxygen/kg oil and peroxide value for refined olive oil ≤ 5 of milliequivalents of active oxygen/kg oil. The obtained results are also agreed with those reported by **El-Sharkawy et al. (1984)** who determined the peroxide values for three olive oil varieties; Shemlali, Mission and Frantio, they found that their values ranged between 2.53–6.49, 1.74–3.44 and 2.98–5.84, respectively. Other determinations were conducted by **Khalil (1987); Awatif et al. (1996)**, they reported the peroxide values of 1.03 and 6.0, respectively. Also, **Bonastre et al. (2004)** reported the peroxide values of olive oils as ranged between 3.70–10.00. Whereas, **Pardo et al. (2007)** reported the peroxide values of virgin olive oils obtained from 5 different varieties

Table (4) Peroxide values of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil	Peroxide values of binary admixtures		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	10.80 (EVOO)	10.80 (EVOO)	10.80 (EVOO)
5	10.70	10.72	10.75
10	10.50	10.15	10.60
20	8.30	9.28	9.50
30	7.90	8.63	8.91
40	5.90	7.93	7.70
50	4.80	6.45	7.01
100	0.90 (SO)	0.55 (CO)	3.70 (ROO)

EVOO; extra virgin olive oil

SO; sunflower oil

CO; corn oil

ROO; refined olive oil

(Picual, Cornicabra, Manzanilla de Centro, Arbequina and local) as follows: 4.44, 3.30, 4.54, 3.85 and 5.70, respectively. Meanwhile, **Méndez and Falqué (2007)** reported peroxide values of commercial extra virgin olive oils as ranged between 18.30-19.70. In this respect, **Espínola et al. (2009)** reported peroxide values of virgin olive oil from 6 varieties; Picual 1, Picual 2, Picual 3, Picual 4, Hojiblanca and Arbequina, as follows: 7.96, 10.0, 12.4, 4.99, 7.15 and 6.30, respectively. Whereas, **Ocakoglu et al. (2009); Oueslati et al. (2009)** reported peroxide values of extra virgin and virgin olive oils as ranged between 4.10-17.00. Also, **Bouaziz et al. (2008)** reported peroxide value of refined olive oil as 15.91.

Concerning the peroxide value of sunflower oil, the obtained result agreed with those reported by **Codex Alimentarius Standard (1999); Egyptian Standards (2005b); National Sunflower Association (2009)**, where the peroxide value of sunflower oil ≤ 10. Other investigators **(Mohamed, 1999; Ahmed, 2004)** reported that the peroxide value of sunflower oil ranged between 1.13-2.90. Also, **Abd El-Fattah (2007)** reported that the peroxide value of sunflower oil was 1.5. While, **Manral et al. (2008)** stated the peroxide value of sunflower oil as 0.1.

El-Nikeety (1981) determined the peroxide value of corn oil as ranged between 1.00–2.90. Also, **Ismael (1989)** evaluated the peroxide value of crude corn oil as 0.71. While, it was ≤ 10 as reported by **Codex Alimentarius Standard (1999); Corn Refiners Association (2006).**

As shown in Table (4), the peroxide values of sunflower, corn, and refined olive were 0.90, 0.55, and 3.70, respectively. This indicated that lesser oxidation products were found in these oils, or if these were formed, they were removed during refining process carried out on oils.

In conclusion, the adulteration of extra virgin olive oil with the adulterant oils (sunflower, corn and refined olive oils) can not be detected at any concentrations of these adulterant oils in extra virgin olive oil by measuring the peroxide value of the binary admixtures of extra virgin olive oil with each of these adulterant oils once it has the same limits established by **Codex Alimentarius Standard (2003); IOOC (2008).**

4.1.4- Iodine Value:

The iodine value is defined as a number of grams from iodine which absorbed by 100 grams of oil or fat. It represents one of the parameters that could differentiate between the qualities of oils. The iodine value is used to indicate the degree of unsaturation of oil fatty acids or for measuring the number of double bonds present in the molecule of oil. The higher the iodine value, the higher the unsaturation of the oil.

The iodine values of extra virgin olive oil and its binary admixtures with different concentrations from the adulterant oils (sunflower oil, corn oil and refined olive oil) were determined and expressed as number of grams from iodine which absorbed by 100 grams of oil. The results are presented in Table (5).

The data indicate that the iodine value of extra virgin olive oil was 82.18, this value is the least one compared to those of the adulterant oils in this study. Refined olive oil was in the second order having an iodine value of 90.40, while the other two adulterant oils; sunflower and corn oils were more unsaturated, and thereby, their iodine values were substantially higher than those of olive oils, being 129.18 and 120.39, respectively.

The results obtained of olive oils agree with those reported by **Codex Alimentarius Standard (2003); Egyptian Standard (2005a),** they established the limits of the iodine values of virgin olive oil, refined olive oil and olive oil ranged between 75.00-94.00.

These results agree also with those determined by **El-Sharkawy et al. (1984)** for three olive oil varieties; Shemlali, Mission and Frantio, their iodine values ranged between 83.38–85.82, 79.36-85.85 and 83.92–86.03, respectively. In addition, **Khalil (1987)** evaluated the iodine value of virgin olive oil; it was 80.34. Whereas, **Mohamed (1999)** reported that the iodine values of olive oils obtained from different areas were in the range between 81.39–84.85. Also, **Hendl et al. (2001)** reported the iodine value of olive oil as 81.30.

Table (5) Iodine values of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil	Iodine values of binary admixtures		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	82.18 (EVOO)	82.18 (EVOO)	82.18 (EVOO)
5	82.35	83.60	82.50
10	84.48	84.03	84.60
20	88.25	87.62	86.30
30	94.37	94.07	87.50
40	97.37	97.52	88.60
50	99.50	99.88	89.80
100	129.18 (SO)	120.39 (CO)	90.40 (ROO)

EVOO; extra virgin olive oil

SO; sunflower oil

CO; corn oil

ROO; refined olive oil

Other investigators; **Méndez and Falqué (2007)** found that the iodine values of commercial extra virgin olive oils ranged between 82.41-87.93.

Concerning the iodine value of the adulterant sunflower seed oil under investigation, it fell in the range between 118.00-141.00 reported by **Codex Alimentarius Standard (1999); Egyptian Standard (2005b)**, the value is supported by **Ahmed (2004)** who reported that the iodine value of sunflower oil ranged between 83.00–144.00. Whereas, it was nearby the values (126.72, 126.44) obtained by **Abd El-Fattah (2007); Manral et al. (2008)**, respectively, and lower than the values (133.00, 135.00) reported by **De Greyta et al. (1998); Mohamed (1999)**, respectively.

Regarding the obtained iodine value of the adulterant corn oil in this study, it is already compatible with the permitted range (103.00-135.00) as reported by **Codex Alimentarius Standard (1999); Corn Refiners Association (2006).** The value is also in line with that value obtained by **El-Nikeety (1981)** who observed that the iodine value of corn oil ranged between 105.00–124.00; and also is agreed with the values of 119.57, 124.00 as reported by **Ismael (1989); De Greyta et al. (1998)**, respectively.

Other investigators; **Barthus and Poppi (2001)** carried out a study to determine the total unsaturation and iodine values of vegetable oils since the iodine value is related to the measurement of the total amount of unsaturation of the oils. They found the iodine values of olive oil as 84.00, sunflower oil as 118.00 and corn oil as 114.00. These reported iodine values were nearby the iodine values tabulated in Table (5) obtained in this study.

On the other hand, **Hendl et al. (2001)** carried out a study to determine the iodine values of vegetable oils. They found that the iodine values were as: 81.30 for olive oil, 120.00 for sunflower oil and 123.50 for corn oil.

Also, **Yang et al. (2005)** reported the iodine values of olive oil as ranged between 83.20-89.00 and corn oil as ranged between 120.78-127.82. In this respect, **Muik et al. (2007)** found that the iodine values for olive, sunflower and corn oils were as: 80.00, 123 and 128, respectively. These reported results for different methods of analysis agreed with the obtained results in this study shown in Table (5).

Concerning the adulteration of extra virgin olive oil with the adulterant sunflower oil, the results in Table (5) demonstrated that although the addition of different concentrations of 5, 10, 20, 30, 40, and 50% from the adulterant sunflower oil to extra virgin olive oil showed gradual increases in the iodine values of the blends starting from pure extra virgin olive oil till pure sunflower oil, these increased iodine values were still present inside the range (75.00-94.00) of the iodine value of extra virgin olive oil reported by **Codex Alimentarius Standard (2003); Egyptian Standard (2005a)** till the adulterant sunflower oil concentration reached 30% which heighten the iodine value of the blend reaching 94.37 and made it out of the permitted level of 94.00 stipulated by **Codex Alimentarius Standard (2003); Egyptian Standard (2005a).**

Thus, starting from this concentration (30%) upwards higher concentration of the adulterant sunflower oil, the adulteration can be revealed by shifting the iodine value of the blend to higher value than 94.00.

Regarding, the adulteration of extra virgin olive oil with the adulterant corn oil, the results in Table (5) demonstrated that although the addition of the different concentrations of 5, 10, 20, 30, 40, and 50% from the adulterant corn oil to extra virgin olive oil showed gradual increases in the iodine values of the blends starting from the pure extra virgin olive oil till pure corn oil, these increased iodine values were still present inside the range (75.00-94.00) of the iodine value of extra virgin olive oil reported by **Codex Alimentarius Standard (2003); Egyptian Standard (2005a)** till the adulterant corn oil concentrations reached 20% in the studied binary admixtures that did not show any shift outside the maximum level of the iodine value of extra virgin olive oil as reported by **Codex Alimentarius Standard (2003); Egyptian Standard (2005a)** which is 94.00, because the recorded iodine value of the blend was 87.62 that being not come more than 94.00, whereas, the addition of higher concentration than 20% of the adulterant corn oil (30%) made the blend out of the range because it gave iodine value of 94.07 which is greater than the maximum permitted iodine value of extra virgin olive oil as reported by **Codex Alimentarius Standard (2003); Egyptian Standard (2005a).**

Thus, initiation from this concentration (30%) upwards higher concentrations of the adulterant corn oil, the adulteration can be revealed by shifting the iodine value of the blend to a higher value than 94.00.

In the last case concerning the adulteration of extra virgin olive oil with the adulterant refined olive oil, results in Table (5) demonstrated that although the addition of the different concentrations of 5, 10, 20, 30, 40, and 50% from the adulterant refined olive oil to extra virgin olive oil showed gradual increases in the iodine values of the blends starting from the pure extra virgin olive oil till pure refined olive oil, these increased values were still present inside the range (75.00-94.00) of the iodine value of extra virgin olive oil reported by **Codex Alimentarius Standard (2003); Egyptian Standard (2005a).**

Thus, the adulteration of extra virgin olive oil with the adulterant refined olive oil can not be detected using the iodine value parameter at any concentration of the adulterant refined olive oil since it has iodine value inside the same range of extra virgin olive oil.

In conclusion, one can detect the adulteration of extra virgin olive oil with the adulterant oils under investigation (sunflower oil, corn oil and refined olive oil) by the determination of iodine value in blends of 30% upward higher concentrations of the adulterant sunflower oil, and in blends of 30% upward higher concentrations of the adulterant corn oil, whereas, the adulteration with refined olive oil can not be detected using the iodine value parameter at any concentration.

4.2- Ultraviolet (UV) Spectroscopic Characteristics:

The spectrophotometric measurement is one of the physical methods which is particularly useful in composition studies on fats and oils and in the analysis and identification of fatty materials. It had been employed for studying and following chemical reactions of fatty materials, especially isomerization, polymerization, oxidation and the determination of rancidity in oils. Unsaturated fatty materials absorb light in the ultraviolet region of the spectrum; this region is between about 200 and 400 nm. In the case of non-conjugated and saturated materials, the absorption is weak and cannot be used for analytical purposes. When linoleic and linolenic acid or more highly unsaturated fatty acids are oxidized to form hydroperoxides, the double bonds in the oils become conjugated. The linoleic and linolenic acids are isomerized to conjugateddienoic and trienoic acids, respectively. The hydroperoxides and the conjugated diene which may result from its decomposition show a strong absorption band at about 232 nm, while conjugated triene and the secondary oxidation products, particularly diketones, show an absorption band at 270 nm.

Conjugated diene absorbencies at 232 nm, conjugated triene absorbencies at 270 nm and ΔK of extra virgin olive oil in binary admixtures with the different adulterant oils (sunflower, corn, and refined olive oils) at various concentrations are listed in Tables (6, 7, 8, 9 and 10).

From Table (6), it appears that K_{232} has the value of 1.151 for extra virgin olive oil which agrees with **Codex Alimentarius Standard (2003); IOOC (2008)**, they reported a trade standard values of absorbency of extra virgin olive oil in UV as follows; $K_{232} \leq 2.50$, $K_{270} \leq 0.22$, $\Delta K \leq 0.01$. From the same table, it is noticed that the values of K_{232} for pure sunflower, corn and refined olive oils were 1.516, 1.919 and 1.647, respectively. Also, the values of K_{232} for the binary admixtures of adulterant oils with extra virgin olive oil at any concentrations were inside the limits of **Codex Alimentarius Standard (2003); IOOC (2008)**, and so these values will not show any significance in the detection of adulteration of extra virgin olive oil with adulterant sunflower, corn and refined olive oils.

As shown in Table (7), the value of K_{270} is 0.131 for extra virgin olive oil which agrees with those reported by **Codex Alimentarius Standard (2003); IOOC (2008)**. However, the values of K_{270} for pure sunflower, corn and refined olive oils were considerably higher than that of extra virgin olive oil being 0.724, 1.027 and 0.984, respectively.

The small value in absorbency at 270 nm of extra virgin olive oil might be further contributed to its low concentration of linoleic acid coupled with surplus oxidative stability of this oil, and subsequently low relative rates of formation of ketonic and aldehydic oxidative products, as well as low formation of conjugated trienes. While, the more absorption at 270 nm caused by the other studied oils which exceeded that of extra virgin olive oil, might be attributed to their higher oxidative degradation of the polyunsaturated linoleate present in these oils at higher concentrations resulting in the formation of conjugated trienoic and ketodienes. The excess UV absorbance could be also explained by the higher amount of activated methylene groups in polyunsaturated fatty acids in these oils than in extra virgin olive oil which are oxidized to form secondary oxidative products.

The obtained results in Table (7) revealed that the UV absorbency at 270 nm of extra virgin olive oil gradually increased with stepping up the concentration of the different adulterant oils in its binary admixtures. It was clearly noticed that the adulteration with adulterant sunflower oil will be detected easily by UV absorbency at 270 nm because it showed a shift out of the range of **Codex Alimentarius Standard (2003); IOOC (2008)**, at 20% upward its higher concentrations with extra virgin olive oil. Similarly, corn oil can be show up if present at the concentration of 5% upward higher concentrations in the binary admixtures with extra virgin olive oil. Also, refined olive oil can be detected if present at the concentration of 10% upward higher concentrations in the binary blends with extra virgin olive oil.

ΔK values of extra virgin olive oil in binary admixtures with adulterant studied oils at various concentrations were tabulated in Tables (8, 9 and 10).

The ΔK was calculated using the UV absorbencies at 268, 262 and 274 nm as follows:

$$\Delta K = [Abs_{268} - (Abs_{262} + Abs_{274})/2]$$

ΔK is useful for readily classifying olive oil quality according to the following values: ΔK for extra virgin olive oil ≤ 0.01, ΔK for virgin olive oil ≤ 0.01, and ΔK for refined olive oil ≤ 0.16 as stipulated by **Codex Alimentarius Standard (2003); IOOC (2008)**.

From Tables (8, 9 and 10), it is apparent that ΔK had the value of 0.0055 for extra virgin olive oil which agree with the aforementioned permitted level stipulated by **Codex Alimentarius Standard (2003); IOOC (2008)**.

The obtained results in this study agree with those reported by **Bilancia et al. (2007)** for extra virgin olive oils obtained from Coratina cultivar which had the following values of K_{232}, K_{270} and ΔK: 1.388, 0.130, and 0.000, respectively.

In addition, **Manai et al. (2008)** evaluated K_{232}, K_{270} and ΔK for virgin olive oils obtained from six cultivars planted over 13 years ago in the Experimental Station of Olive Institute in Sfax (South of Tunisia) as follows: 1.57, 0.089, 0.005; 1.24, 0.085, 0.002; 1.71, 0.192, 0.005; 1.35, 0.155, 0.001; 1.68, 0.150, 0.001; 1.49, 0.133, 0.002, respectively.

In this respect, **Allalout et al. (2009)** reported the values of K_{232}, K_{270}, ΔK for virgin olive oils obtained from Super Intensive Spanish and Greek varieties grown in northern Tunisia; Arbequina, Arbequina I-18 and Arbosana from Spain and Koroneiki from Greece, as following: 1.46, 0.08, 0.004; 1.49, 0.09, 0.002; 2.24, 0.16, 0.003; 1.00, 0.20, 0.001 for the aforementioned varieties, respectively.

The values of ΔK for pure sunflower, corn and refined olive oils were 0.100, 0.07 and 0.117, respectively, being higher than that of extra virgin olive oil.

Table (6) Conjugated diene absorbencies (at 232 nm) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil %	Abs $_{232}$ nm of binary admixtures		
	EVOO + SO	EVOO + CO	EVOO + ROO
0	1.151 (EVOO)	1.151 (EVOO)	1.151 (EVOO)
5	1.241	1.250	1.163
10	1.244	1.346	1.170
20	1.307	1.522	1.249
30	1.360	1.522	1.269
40	1.470	1.647	1.279
50	1.478	1.723	1.425
100	1.516 (SO)	1.919 (CO)	1.647 (ROO)

EVOO; extra virgin olive oil

SO; sunflower oil

CO; corn oil

ROO; refined olive oil

Table (7) Conjugated triene absorbencies (at 270 nm) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil	Abs $_{270}$ nm of binary admixtures		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	0.131 (EVOO)	0.131 (EVOO)	0.131 (EVOO)
5	0.180	0.223	0.213
10	0.191	0.324	0.245
20	0.221	0.380	0.380
30	0.280	0.426	0.481
40	0.393	0.482	0.682
50	0.407	0.549	0.727
100	0.724 (SO)	1.027 (CO)	0.984 (ROO)

EVOO; extra virgin olive oil

SO; sunflower oil

CO; corn oil

ROO; refined olive oil

Table (8) Determination of ΔK of extra virgin olive oil in binary admixtures with adulterant sunflower oil at various concentrations

Adulterant oil	Absorbency at specific wavelength			ΔK
%	Abs $_{262}$ nm	Abs $_{268}$ nm	Abs $_{274}$ nm	
0 (EVOO)	0.0420	0.0360	0.0190	0.0055
5	0.0420	0.0630	0.0300	0.0270
10	0.1420	0.1570	0.0960	0.0380
20	0.0730	0.1170	0.0730	0.0440
30	0.2250	0.2380	0.1460	0.0525
40	0.2030	0.2260	0.1370	0.0560
50	0.2730	0.2780	0.1500	0.0665
100 (SO)	0.3270	0.4900	0.4460	0.1000

EVOO; extra virgin olive oil

SO; sunflower oil

Table (9) Determination of ΔK of extra virgin olive oil in binary admixtures with adulterant corn oil at various concentrations

Adulterant oil	Absorbency at specific wavelength			ΔK
%	Abs $_{262}$ nm	Abs $_{268}$ nm	Abs $_{274}$ nm	
0 (EVOO)	0.0420	0.0360	0.0190	0.0055
5	0.2610	0.2390	0.1760	0.0205
10	0.1300	0.1070	0.1270	0.0215
20	0.2730	0.2470	0.1780	0.0215
30	0.1230	0.0990	0.1190	0.0220
40	0.1300	0.1050	0.1270	0.0230
50	0.3780	0.2470	0.2050	0.0440
100 (CO)	0.4500	0.5680	0.5340	0.0700

EVOO; extra virgin olive oil

CO; corn oil

Table (10) Determination of ΔK of extra virgin olive oil in binary admixtures with adulterant refined olive oil at various concentrations

Adulterant oil %	Absorbency at specific wavelength			ΔK
	Abs $_{262}$ nm	Abs $_{268}$ nm	Abs $_{274}$ nm	
0 (EVOO)	0.0420	0.0360	0.0190	0.0055
5	0.0620	0.0910	0.0890	0.0155
10	0.0590	0.0890	0.0800	0.0295
20	0.1700	0.1060	0.1270	0.0325
30	0.2120	0.2230	0.1490	0.0425
40	0.1900	0.1150	0.1360	0.0580
50	0.2130	0.2850	0.1830	0.0670
100 (ROO)	0.3450	0.4200	0.3410	0.1170

EVOO; extra virgin olive oil

ROO; refined olive oil

Concerning the adulteration of extra virgin olive oil with the adulterant sunflower oil, ΔK values of binary admixtures of extra virgin olive oil with this oil are shown in Table (8) indicating that the ΔK values increased gradually with increasing the concentration of the adulterant sunflower oil in the blends ranging from 0.0055 for extra virgin olive oil, till pure sunflower oil. Fortunately, it was found that ΔK at 5% adulterant sunflower oil in binary admixture with extra virgin olive oil was 0.0270 which made the blend out of the level of **Codex Alimentarius Standard (2003); IOOC (2008)** which implies that ΔK ≤ 0.01 for extra virgin olive oil. These findings mean that it is possible to detect the adulterant sunflower oil at 5% in its binary admixture with extra virgin olive oil.

Similarly, from ΔK values of the binary admixtures of extra virgin olive oil with the adulterant corn oil shown in Table (9), ΔK increased gradually with increasing concentration of the adulterant corn oil in the blends ranging from 0.0055 for extra virgin olive oil, till pure corn oil. It was found that the ΔK at 5% adulterant corn oil in binary admixture with extra virgin olive oil was 0.0205 which made the blend out of the level of **Codex Alimentarius Standard (2003); IOOC (2008)** which implies that ΔK ≤ 0.01 for extra virgin olive oil. Hereupon, it is possible to detect the adulterant corn oil at 5% in its binary admixture with extra virgin olive oil.

Regarding Table (10), the ΔK values of binary admixtures of extra virgin olive oil with the adulterant refined olive oil increased gradually with increasing concentration of adulterant refined olive oil in the blends ranging from 0.0055 for extra virgin olive oil, till pure refined olive oil. The ΔK for 5% adulterant refined olive oil in binary admixture with extra virgin olive oil was 0.0155 which made the blend out of the limit of **Codex Alimentarius Standard (2003); IOOC (2008)** for extra virgin olive oil. As described, it was possible to disclose the adulterant refined olive oil at 5% in its binary admixture with extra virgin olive oil.

In conclusion, adulteration of extra virgin olive oil with adulterant sunflower, corn and refined olive oils could be detected at 5% of adulterant oil added, using ΔK values obtained from UV characteristics.

4.3- Gas Chromatography (GC) Analysis:

Gas chromatography was first developed by lipid analysts, and lipid analysts have been at the forefront in the development of the technique. In recent years, there has been a remarkable improvement in the resolution attainable by gas chromatography thanks to the availability of capillary columns fabricated from fused silica. Gas chromatography is the most frequently used technique for the analysis of fatty acids of oils.

4.3.1- GC Analysis of Fatty Acids:

The fatty acids composition identified of extra virgin olive oil and the binary admixtures with adulterant sunflower, corn and refined olive oils at various concentrations of 5, 10, 20, 30, and 50% were determined using GC. The obtained results are presented in Tables (11, 12 and 13).

Concerning the fatty acids composition of extra virgin olive oil, it is seen that it contained palmitic acid as a major saturated fatty acid, followed by stearic acid; their percentages were 15.86% and 2.40%, respectively. As for the unsaturated fatty acids, it is clear that oleic acid was the most predominant fatty acid among not only unsaturated ones, but also all over the fatty acids identified in extra virgin olive oil. It amounted to 71.09% followed by linoleic acid; 6.21%. ΣSFA was 19.78, ΣUSFA 79.57, the ratios of ΣSFA/ΣUSFA was 0.248, C16:0/C18:2 was 2.55, C18:1/C18:2 was 11.44 and C16:0/C16:1 was 10.10.

These results agree with those reported by **Codex Alimentarius Standard (2003)** with following ranges: $C_{14:0}$: 0.0-0.05, $C_{16:0}$: 7.5–20.0, $C_{16:1}$: 0.3 - 3.5, $C_{17:0}$: 0.0-0.3, $C_{17:1}$: 0.0-0.3, $C_{18:0}$: 0.5-5.0, $C_{18:1}$: 55.0-83.0, $C_{18:2}$: 3.5–21.0, $C_{18:3} < 1$, $C_{20:0}$: 0.0-0.6, $C_{20:1}$: 0.0-0.4, $C_{22:0}$: 0.0-0.2, $C_{24:0}$: 0.0-0.2. Also, the obtained fatty acids composition of extra virgin olive oil agrees with **Egyptian Standards (2005a); IOOC (2008)** and with those reported by **Marini et al. (2004); Morelló et al. (2004); Aguilera et al. (2005); Ollivier et al. (2006); Ben Temime et al. (2006); Marini et al. (2006); Blasi et al. (2007); Matos et al. (2007); D'Imperio et al. (2007); Galtier et al. (2007); Sánchez-Gimeno et al. (2008); Huang and Sumpio (2008); Koprivnjak et al. (2008); Chiavaro et al. (2009).**

Table (11) Fatty acids composition percent of extra virgin olive oil in binary admixtures with adulterant sunflower oil at various concentrations

Fatty Acid	0 (EVOO)	5	10	20	30	40	50	100 (SO)
C 16:0	15.86	13.28	12.84	12.59	11.69	10.44	10.00	7.20
C 16:1	1.57	1.55	1.48	1.43	1.42	1.40	1.19	0.23
C 17:0	0.11	0.04	0.05	0.05	0.05	0.06	0.06	0.15
C 17:1	0.14	0.10	0.09	0.08	0.08	0.07	0.06	0.06
C 18:0	2.40	2.48	2.60	3.00	3.20	3.30	3.50	4.11
C 18:1	71.09	67.96	66.12	61.47	56.97	53.34	48.55	27.78
C 18:2	6.21	12.46	14.93	19.61	25.25	30.40	35.73	59.58
C 18:3	0.49	0.47	0.45	0.43	0.41	0.39	0.37	0.30
C 20:0	0.71	0.21	0.11	0.10	0.09	0.02	0.01	0.14
C 20:1	0.07	0.07	0.07	0.09	0.10	0.11	0.11	0.13
C 22:0	0.40	0.39	0.37	0.37	0.36	0.34	0.32	0.23
C 24:0	0.30	0.28	0.28	0.07	0.04	0.02	0.01	0.00
ΣSFA	19.78	16.68	16.25	16.18	15.43	14.18	13.90	11.83
ΣUSFA	79.57	82.61	83.14	83.11	84.23	85.71	86.01	88.08
ΣSFA/ΣUSFA	0.248	0.202	0.195	0.194	0.183	0.165	0.161	0.134
C16:0/C18:2	2.55	1.06	0.86	0.64	0.46	0.34	0.28	0.12
C18:1/C18:2	11.44	5.45	4.42	3.13	2.25	1.75	1.35	0.46
C16:0/C16:1	10.10	8.57	8.68	8.80	8.23	7.46	8.40	31.30

EVOO; extra virgin olive oil

SO; sunflower oil

4.3.1.1- Detection of adulteration of extra virgin olive oil with adulterant sunflower oil using fatty acids composition:

The percentages of the fatty acids identified of extra virgin olive oil and the admixtures with adulterant sunflower oil at various concentrations of 5, 10, 20, 30, and 50% were determined using GC. The results are tabulated in Table (11). The data indicated that the fatty acids identified for sunflower oil mainly consisted of palmitic acid as a higher saturated fatty acid, it amounted to 7.20% followed by stearic acid; 4.11%. As for unsaturated fatty acids, linoleic acid showed a higher percentage more than that found for oleic acid in contrast to those in extra virgin olive oil. Their concentrations were 59.58% and 27.78% for the above mentioned fatty acids, respectively. Linolenic fatty acid was found in very low concentration being 0.30%. ΣSFA was 11.83, ΣUSFA 88.08, the ratios of ΣSFA/ΣUSFA was 0.134, C16:0/C18:2 was 0.12, C18:1/C18:2 was 0.46 and C16:0/C16:1 was 31.30.

These results agree with those reported by **Codex Alimentarius Standard (1999); Egyptian Standards (2005b)** which had the following ranges: $C_{14:0}$: 0.05-0.2, $C_{16:0}$: 5–7.6, $C_{16:1}$: 0.05–0.3, $C_{17:1}$: 0.05-0.1, $C_{18:0}$: 2.7-6.5, $C_{18:1}$: 14-39.4, $C_{18:2}$: 48.3-74, $C_{18:3}$: 0.05-0.3**,** $C_{20:0}$: 0.1-0.5, $C_{22:0}$: 0.3-1.5, $C_{22:1}$: 0.05-0.3. Also, the obtained fatty acid composition of sunflower oil agrees with those reported by other investigators; **Abd El-Razek (1998)**; **Favier et al. (1998); Quiles et al. (2002); Ahmed (2004); Brodnjak-Vončina et al. (2005); Abd El-Fattah (2007); Smith et al. (2007); Dizge et al. (2009).**

From the same table, it is clear that the addition of different concentrations 5, 10, 20, 30, 40, and 50% from sunflower oil to extra virgin olive oil showed remarkable changes in the fatty acids pattern of the extra virgin olive oil (adulterated olive oil). Palmitic and oleic acids percentages showed gradual decreases as the amount of sunflower oil added to extra virgin olive oil increased. On the other hand, linoleic acid which had a lesser percentages in extra virgin olive oil of 6.21%, showed a gradual increases with increasing the adulterant sunflower oil concentrations. Therefore, the total saturated fatty acids showed a gradual increase. From the data reported in **Codex Alimentarius Standard (2003)** for extra virgin olive oil, the range of linoleic acid should be ranged from 3.5 – 21.0%. Hereupon, the blends contained up to 20% adulterant sunflower oil was already included as extra virgin olive oil because linoleic acid concentration percent was 19.61% at 20% of adulterant sunflower oil, since this value is still present inside the range of **Codex Alimentarius Standard (2003)** for extra virgin olive oil. With consequent addition of the adulterant sunflower oil beyond 20%, the concentration of linoleic acid increased, it reached 25.25% at 30% of the adulterant sunflower oil which is out of the range reported by **Codex Alimentarius Standard (2003)** for extra virgin olive oil.

It is also noticed that the concentration of oleic acid was 53.34% at 40% adulterant sunflower oil which is out of the range of **Codex Alimentarius Standard (2003)** for extra virgin olive oil, the range of oleic acid should be ranged from 55.0 – 83.0%.

It is concluded that the fatty acid composition of any oil sample identified by GC technique could be used as a method for detection of adulterated extra virgin olive oil with sunflower oil at an added percentage of 30% and more from sunflower oil, while the levels below this added percentage can not be uncovered by the fatty acid composition.

4.3.1.2- Detection of adulteration of extra virgin olive oil with adulterant corn oil using fatty acids composition:

The percentages of the fatty acids identified of extra virgin olive oil and its binary admixtures with adulterant corn oil at various concentrations of 5, 10, 20, 30, and 50% were determined using GC. The results are presented in Table (12). From this table, it could be seen that corn oil contained palmitic acid as a major saturated fatty acid; its percentage was amounted to 12.0%. As for the unsaturated fatty acids, linoleic acid was the predominant one followed by oleic acid; their concentrations were 54.0% and 30.22%, respectively.

Table (12) Fatty acids composition percent of extra virgin olive oil in binary admixtures with adulterant corn oil at various concentrations

Fatty Acid	0 (EVOO)	5	10	20	30	40	50	100 (CO)
C 16:0	15.86	15.01	14.54	14.21	13.86	13.36	13.14	12.00
C 16:1	1.57	1.54	1.38	1.34	1.25	1.23	1.11	0.20
C 17:0	0.11	0.09	0.08	0.07	0.07	0.06	0.06	0.10
C 17:1	0.14	0.09	0.08	0.08	0.07	0.06	0.05	0.00
C 18:0	2.40	2.00	1.98	1.97	1.95	1.85	1.14	1.98
C 18:1	71.09	69.40	65.41	62.72	59.00	54.23	51.79	30.22
C 18:2	6.21	10.12	14.48	17.89	22.15	27.24	30.65	54.00
C 18:3	0.49	0.77	0.81	0.81	0.89	0.90	0.94	0.98
C 20:0	0.71	0.48	4.47	0.45	0.44	0.43	0.39	0.27
C 20:1	0.07	0.08	0.09	0.10	0.12	0.13	0.14	0.15
C 22:0	0.40	0.29	0.15	0.15	0.13	0.13	0.11	0.10
C 24:0	0.30	0.10	0.09	0.08	0.08	0.04	0.03	0.00
ΣSFA	19.78	17.97	17.31	16.93	16.53	15.87	14.87	14.45
ΣUSFA	79.57	82.00	82.25	82.94	83.38	83.79	84.68	85.55
ΣSFA/ΣUSFA	0.248	0.219	0.210	0.204	0.198	0.189	0.175	0.168
C16:0/C18:2	2.55	1.483	1.00	0.79	0.625	0.490	0.428	0.222
C18:1/C18:2	11.44	6.85	4.51	3.50	2.66	1.99	1.689	0.559
C16:0/C16:1	10.10	9.75	10.54	10.60	11.09	10.86	11.84	60.00

EVOO; extra virgin olive oil
CO; corn oil

While linolenic acid was identified in a rare percentage being 0.98%. ΣSFA was 14.45, ΣUSFA 85.55, the ratios of ΣSFA/ΣUSFA was 0.168, C16:0/C18:2 was 0.222, C18:1/C18:2 was 0.559 and C16:0/C16:1 was 60.0. These results agree with those reported by **Codex Alimentarius Standard (1999); Corn Refiners Association (2006).** Also, the obtained fatty acid composition of corn oil agrees with those reported by **Ismael (1989)**; **Brodnjak-Vončina et al. (2005); Smith et al. (2007).**

Table (12) shows that the addition of different concentrations (5, 10, 20, 30, 40, and 50%) from corn oil to extra virgin olive oil changed the pattern and percentages of extra virgin olive oil fatty acids. Palmitic and oleic acids showed gradual decreases as percentage of corn oil added increased, but still present inside range of **Codex Alimentarius Standard (2003)** for extra virgin olive oil, the above two fatty acids should be ranged from 7.5–20.0% for palmitic acid and from 55.0–83.0% for oleic acid. With consequent addition of adulterant corn oil till 40%, the blend come to be out of the range of **Codex Alimentarius Standard (2003)** for extra virgin olive oil because at this added concentration the oleic acid percent in the blend decreased reaching 54.23% being lesser than the permitted minimum level reported by **Codex Alimentarius Standard (2003)** for extra virgin olive oil which was 55.0%. This means that it is possible to detect adulteration of extra virgin olive oil by corn oil at 40% concentration by the means of oleic acid percent in binary admixtures.

Meanwhile, linoleic percentages showed a gradual increases as the ratio of corn oil added increased till it reached 22.15% at 30% of adulterant corn oil which made the blend out of the range of **Codex Alimentarius Standard (2003)** for extra virgin olive oil, which had range of linoleic acid between 3.5–21.0%. As described, it is possible to detect the adulteration of extra virgin olive oil by adulterant corn oil at 30% concentration by the means of linoleic acid percent in mixtures.

From table (12) also, it could be seen that ΣSFA in extra virgin olive oil decreased with the increasing percentage of corn oil. In contrary, the ΣUSFA were increased gradually.

4.3.1.3- Detection of adulteration of extra virgin olive oil with adulterant refined olive oil using fatty acids composition:

Table (13) illustrates the percentages of the fatty acids which identified by GC technique of extra virgin olive oil in binary admixtures with adulterant refined olive oil at various concentrations.

From this table, it could be seen that the refined olive oil contained saturated fatty acids. Palmitic acid was the most predominant saturated fatty acid in refined olive oil which amounted to 18.73%, while stearic acid was in the second order and amounted to 5.05% being higher than those found in extra virgin olive oil. It is also noticed that the unsaturated fatty acids of refined olive oil was mainly consisted of oleic acid as a major unsaturated one (56.08%), followed by linoleic acid (16.22%), while linolenic acid was determined as 1.76% (higher than that found in extra virgin olive oil). ΣSFA was 24.55, ΣUSFA 75.41, the ratios of ΣSFA/ΣUSFA was 0.325, C16:0/C18:2 was 1.154, C18:1/C18:2 was 3.45 and C16:0/C16:1 was 13.87. These results agree with that reported by **Codex Alimentarius Standard (2003)** in which the percent of linolenic acid should be less than 1.0.

As shown in Table (13), with consequent addition of adulterant refined olive oil, there were consequence increases in the linolenic acid percent in the binary admixture till reached 0.96% when 10% adulterant refined olive oil was added and then it jumped to 1.20% with raising the concentration of adulterant refined olive oil to 20% which made the blend out of the range reported by **Codex Alimentarius Standard (2003)** in which the linolenic acid should be less than 1.0%. This means that it is possible to detect the adulteration of extra virgin olive oil by adulterant refined olive oil at 20% concentration and more by the means of linolenic acid percent in the mixtures.

Data in Table (13) indicated also that addition of different concentrations of refined olive oil of 5, 10, 20, 30, 40, and 50% showed noticeable decreases in oleic acid with increasing the ratio of refined olive oil added to the extra virgin olive oil but it still inside the range reported

Table (13) Fatty acids composition percent of extra virgin olive oil in binary admixtures with adulterant refined olive oil at various concentrations

Fatty Acid	0 (EVOO)	5	10	20	30	40	50	100 (ROO)
C 16:0	15.86	15.97	16.10	16.12	16.13	16.17	16.47	18.73
C 16:1	1.57	1.49	1.42	1.39	1.36	1.21	1.10	1.35
C 17:0	0.11	0.09	0.07	0.04	0.03	0.02	0.02	0.00
C 17:1	0.14	0.11	0.09	0.07	0.05	0.03	0.01	0.00
C 18:0	2.40	3.55	3.70	3.91	4.54	4.96	5.03	5.05
C 18:1	71.09	69.26	67.46	65.21	63.58	61.46	60.16	56.08
C 18:2	6.21	7.47	9.04	10.88	11.61	13.66	14.54	16.22
C 18:3	0.49	0.76	0.96	1.20	1.44	1.49	1.67	1.76
C 20:0	0.71	0.60	0.49	0.44	0.44	0.19	0.10	0.00
C 20:1	0.07	0.05	0.03	0.03	0.02	0.01	0.00	0.00
C 22:0	0.40	0.42	0.45	0.51	0.66	0.69	0.71	0.77
C 24:0	0.30	0.21	0.18	0.16	0.12	0.10	0.07	0.00
ΣSFA	19.78	20.84	20.99	21.18	21.92	22.13	22.40	24.55
ΣUSFA	79.57	79.14	79.00	78.78	78.06	77.86	77.48	75.41
ΣSFA/ΣUSFA	0.248	0.263	0.265	0.269	0.280	0.284	0.289	0.325
C16:0/C18:2	2.55	2.137	1.78	1.48	1.389	1.183	1.133	1.154
C18:1/C18:2	11.44	9.27	7.46	5.99	5.47	4.49	4.13	3.45
C16:0/C16:1	10.10	10.72	11.34	11.60	11.86	13.36	14.97	13.87

EVOO; extra virgin olive oil

ROO; refined olive oil

by **Codex Alimentarius Standard (2003)**. Meanwhile, palmitic (the major saturated fatty acid), linoleic and linolenic acids percentages were increased with the increasing of adulterant ratios.

Therefore, it could be concluded from the data present in Tables (11, 12 and 13), that oleic, linoleic, and linolenic acids percentages obtained by GC analysis could be used as a tool to detect the adulteration of extra virgin olive oil in binary admixtures with sunflower oil, corn oil and refined olive oil at added concentrations of 30%, 30% and 20% for the mentioned three adulterant oils, respectively.

4.3.2- Total Unsaponifiable Matter:

Total unsaponifiable matter composition percents of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower, corn, and refined olive oils) at various concentrations of 5, 10, 20, 30, and 50% were determined. The obtained results are presented in Table (14). From this table it is observed that the extra virgin olive oil had 1.48% as total unsaponifiable matter. These results agree with those reported by **Codex Alimentarius Standard (2003); Egyptian Standards (2005a); IOOC (2008)** which had the range of ≤ 15 g/Kg (or 1.5%) for olive oils. Concerning pure sunflower, corn and refined olive oils, they showed total unsaponifiable matter percent of 1.18, 1.15 and 1.35%, respectively.

Concerning sunflower oil, the obtained results were inside the range of **Codex Alimentarius Standard (1999); Egyptian Standards (2005b); National Sunflower Association (2009)**, in which the total unsaponifiable matter of sunflower oil is ≤ 15 g/kg (or ≤ 1.50%)

For corn oil, the obtained results were inside the range of **Codex Alimentarius Standard (1999); Corn Refiners Association (2006)**, in which the total unsaponifiable matter of corn oil is ≤ 28 g/Kg (or ≤ 2.8%).

With consequent addition of each of the 3 adulterant oils, obtained values of total unsaponifiable matter percent were still present inside ranges of **Codex Alimentarius Standard (2003); Egyptian Standards (2005a); IOOC (2008)**. Hereupon, adulteration of extra virgin olive oil could not be detected at any concentrations of these adulterant oils.

Table (14) Total unsaponifiable matter composition percents of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil	Total unsaponifiable matter composition percents of binary admixtures		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	1.48 (EVOO)	1.48 (EVOO)	1.48 (EVOO)
5	1.43	1.36	1.48
10	1.41	1.33	1.47
20	1.39	1.29	1.45
30	1.35	1.25	1.44
40	1.30	1.21	1.42
50	1.25	1.19	1.39
100	1.18 (SO)	1.15 (CO)	1.35 (ROO)

EVOO; extra virgin olive oil

SO; sunflower oil

CO; corn oil

ROO; refined olive oil

4.4- Fourier Transform Infra Red Spectroscopic Characteristics:

4.4.1- FTIR Spectral Data Analysis:

Every chemical compound has its own characteristic IR spectrum. The IR spectrum contains the entire information about the molecular structure of the investigated sample. The main problem is the assignment of experimental spectral bands. In addition to fundamental vibration bands, very often so-called combination and overtone bands are present. Fermi resonance can cause intensity changes and frequency shifts of the bands involved. Intermolecular interactions (such as hydrogen bonding) can cause additional bands. Furthermore, the influences of solvents, temperature and pressure have to be considered. The most convenient tool for identification of molecules from their vibrational spectrum is spectral databases. The matching process is very much accelerated by computerized search programs. If an exact match cannot be found, these programs usually list the reference compounds that numerically match the unknown spectrum very closely. A more chemical approach is the evaluation of characteristic or group frequencies. Some chemical groups exhibit very characteristic bands regardless of the kind of molecule in which they are included. The group frequency approach is very useful for structural analysis. From the frequencies and intensities of spectral bands it is possible to predict what kind of chemical groups are present in the molecule, how they are connected to other groups and finally the structure of the molecule under investigation **(Gauglitz and Vo-Dinh, 2003).**

According to **Gauglitz and Vo-Dinh (2003)**; FTIR spectral region may be classified into 3 regions as following:

1- The region from 4000 to 1400 cm^{-1}: This region comprises stretching vibrations involving movements of light atoms (molar mass below 20 g mol^{-1}). O–H and N–H stretching bands are located in the region from 3700 to 2500 cm^{-1}. These groups very often participate in the formation of hydrogen bond complexes. The formation of such complexes results in red shifted and very broad spectral bands. C–H stretching bands are found in the 3300-2800 cm^{-1} spectral range. Bands in the 2700-1850 cm^{-1} spectral region usually belong to C≡C, C≡N, N≡N or some groups containing hydrogen and a heavier atom (S−H, P−H and Si−H). The 1950-1450 cm^{-1} region exhibits IR absorption from a wide variety of double-bonded chemical groups, in particular C=O. This region is of particular importance for investigations of biological molecules. Conjugation, ring size, hydrogen bonding, steric and electronic effects often result in significant shifts in absorption frequencies.

2- The region from 1400-900 cm^{-1}: This is called the fingerprint region. Many chemical groups with single bonds have group frequencies in this region. These vibrations usually couple very strongly, i.e. particular bands in this region can hardly be attributed to a single chemical bond or group. On the other hand, bands caused by complex interacting vibrations constitute a unique fingerprint for each compound. If two spectra exhibit identical fingerprint patterns in this region, the corresponding samples are generally considered to be identical.

3- The region from 900 to 400 cm^{-1}: Some characteristic bands of aromatics occur in this region. These bands are due to aromatic C–H out-of-plane bending vibrations. The absence of absorption bands in the 900 to 650 cm^{-1} region usually indicates the lack of aromatic rings in the molecule under investigation. Some organic molecules containing halogen atoms can also contribute in this region.

4.4.2- Detection of adulteration of virgin olive oil by FTIR Spectroscopy:

Figure (1) represents the FTIR absorption spectra of mixtures of pure oils (sunflower, corn, extra virgin olive and refined olive oils). Figures (2, 3 and 4) represent the FTIR transmittance spectra of the binary admixtures of extra virgin olive oil with different concentrations of each of sunflower, corn and refined olive oils, respectively. The individual FTIR absorption spectra of pure oils (extra virgin olive, sunflower, corn and refined olive oils) are represented in Figures (5, 6, 7 and 8), respectively. Table (15) represents a comparison of FTIR spectral data of pure oils (extra virgin olive, sunflower, corn and refined olive oils).

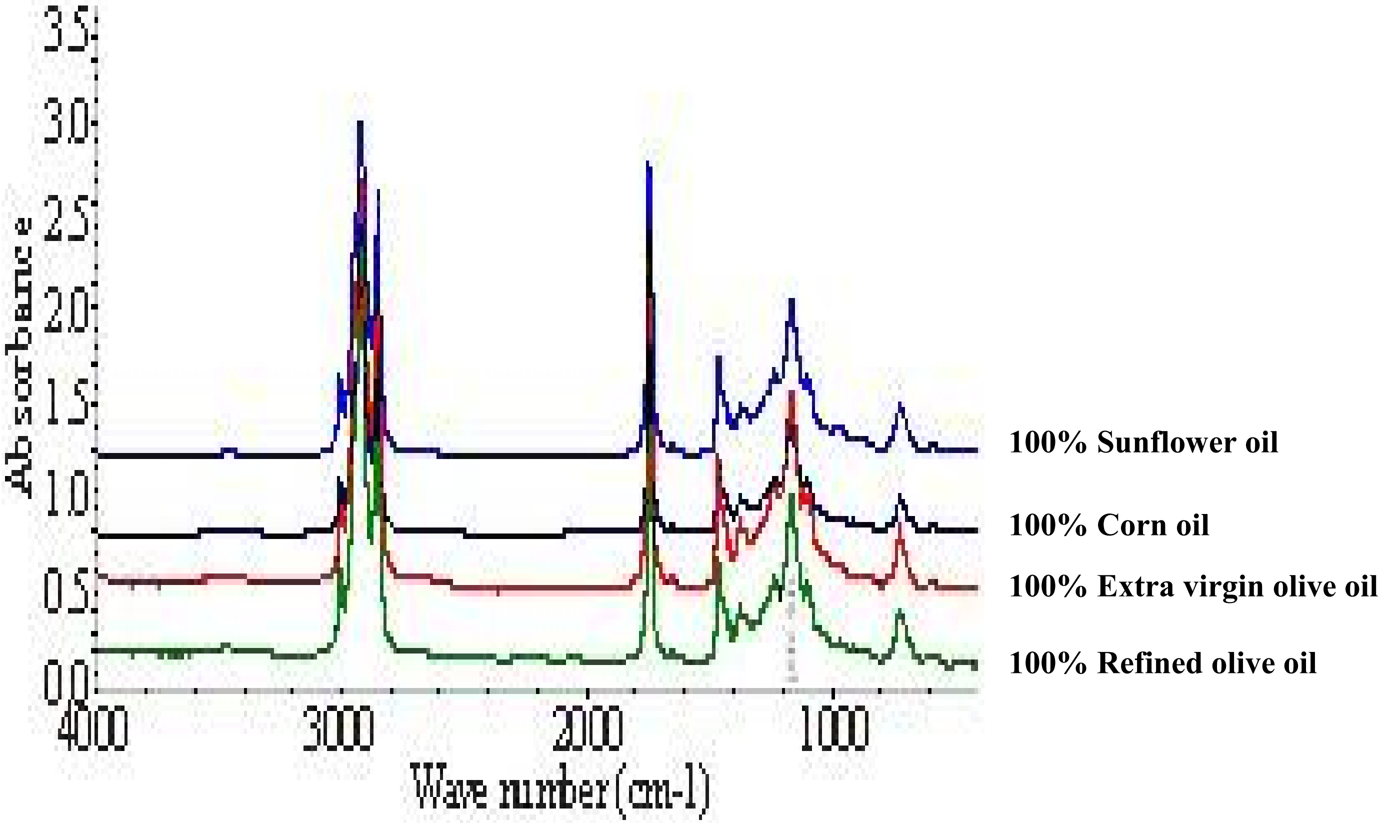

Figure (1) Typical FTIR absorption spectra (4000-400 cm^{-1}) of pure oils (sunflower, corn, extra virgin olive and refined olive oils)

It is well known that every oil or fat differs in composition, length and unsaturated degree of the fatty acids as well as their positions in the chain. The triglyceride, which is a major component in edible oils and fats, was dominant in the spectra. The major peaks that represent triglyceride functional groups could be observed around 2925 cm^{-1} assigned to C–H stretching (asymmetry), 2854 cm^{-1} assigned to C–H stretching (symmetry), 1747 cm^{-1} assigned to C=O stretching, 1463 cm^{-1} assigned to C–H bending (scissoring), 1163 cm^{-1} assigned to C–O stretching and C–H bending, and 722 cm^{-1} assigned to C–H bending (rocking). There was a very weak peak around 1653 cm^{-1} in FTIR spectra, which is assigned to C=C stretching (*cis*). The region selected between 1400 and 1800 cm^{-1} mostly represents the combination of C–H bending, C=O stretching, and C=C stretching and hence is directly related to unsaturated C=C bond. It is demonstrated that vibrations of C–H bending, C=O stretching, and C=C stretching played a very important role in discriminant analysis **(Guillén and Cabo, 1997; Muik et al., 2007).**

As shown in Figure (1), the entire range of spectra looks almost similar for all oils unless one observes very closely. This was due to their similar chemical composition. Most of the spectral information used for the discriminant analysis is compressed in the wavenumber regions of 3100–2800 cm^{-1} and 1800–900 cm^{-1}. It is clear that the prominent peaks are due to C–H stretching in the wavenumber region of 3100–2800 cm^{-1}, C=O stretching in the region of 1800–1700 cm^{-1} and C–O–C stretching, C–H bending in the region of 1400–900 cm^{-1}. The major result of the method developed for the discriminant analysis relies on the exploitation of these small changes in the regions of interest. There are certain variations between the spectra of extra virgin olive oil and sunflower oil in the fingerprint wavenumber region of 1200–800 cm^{-1}. The samples were classified into two groups: adulterated and pure extra virgin olive oils, and the discriminant analysis was applied to both in the wavenumber regions of 3100–2800 cm^{-1} and 1800–900 cm^{-1}. These results showed an excellent agreement with those reported by **Tay et al. (2002).**

From Figures (2, 3 and 4), one can deduce that the major peaks that represent the triglyceride functional groups could be observed around 2925 cm^{-1} due to C–H stretching (asymmetry), 2854 cm^{-1} due to C–H stretching (symmetry), 1747 cm^{-1} due to C=O stretching, 1463 cm^{-1} due to C–H bending (scissoring), 1163 cm^{-1} due to C–O stretching and C–H bending, and 722 cm^{-1} due to C–H bending (rocking). There was a very weak peak around 1653 cm^{-1} in FTIR spectra, which is attributed to the presence of C=C stretching (*cis*). These obtained results agreed with those reported by **Yang et al. (2005); Vlachos et al. (2006).**

These aforementioned major predominant peaks were present in all samples but with varying intensities. Also, there were probably some wavenumber shifts for the same chemical groups in different samples which are attributed to many acceptable reasons that will not affect the identity of the chemical groups as described formerly by **Gauglitz and Vo-Dinh (2003).**

When considering carefully the Figures (5, 6, 7 and 8) which represent FTIR Absorption Spectra of pure extra virgin olive; pure sunflower; pure corn and pure refined olive oils, one can find valuable differences in their FTIR spectral data concerning the intensities of predominant peaks and the waveunmber shifts due to different oil compositions.

Comparison of FTIR spectral data for extra virgin olive, pure sunflower, pure corn and pure refined olive oils is shown in Table (15). From this table, it is easily deduced that the FTIR spectra of extra virgin olive oil were dominated by peaks around 3471, 3005, 2925, 2854, 2679, 1747, 1653, 1463, 1417, 1402, 1376, 1238, 1163, 1118, 1097, 1031, 912 and 722 cm^{-1}. Absorbencies at 2925 and 2854 cm^{-1} were due to bands arising from CH_2 stretching vibrations; asymmetric and symmetric, respectively. The major peak at 1747 cm^{-1} aroused from C=O stretching vibrations; the bands at 1463 and 1376 cm^{-1} aroused from CH_2 and CH_3 scissoring vibration, while those at 1238, 1163, 1118, 1097 were associated with C–O stretching vibration. A peak around 912 cm^{-1} was

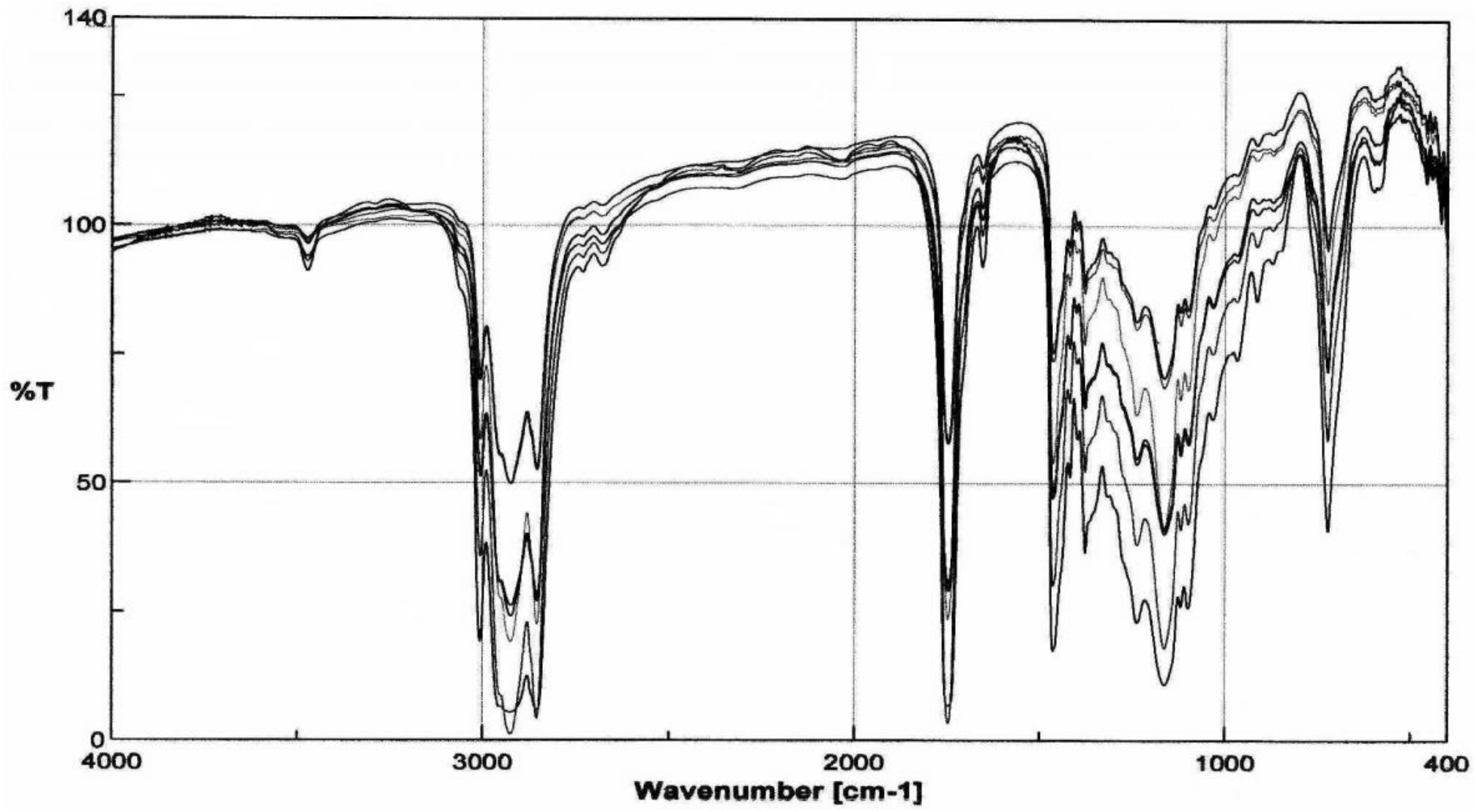

Figure (2) Typical FTIR transmittance spectra of pure extra virgin olive oil (top) mixed with different ratios (0, 5, 10, 20, 30, 40, 50 and downwards till 100%) of sunflower oil (bottom).

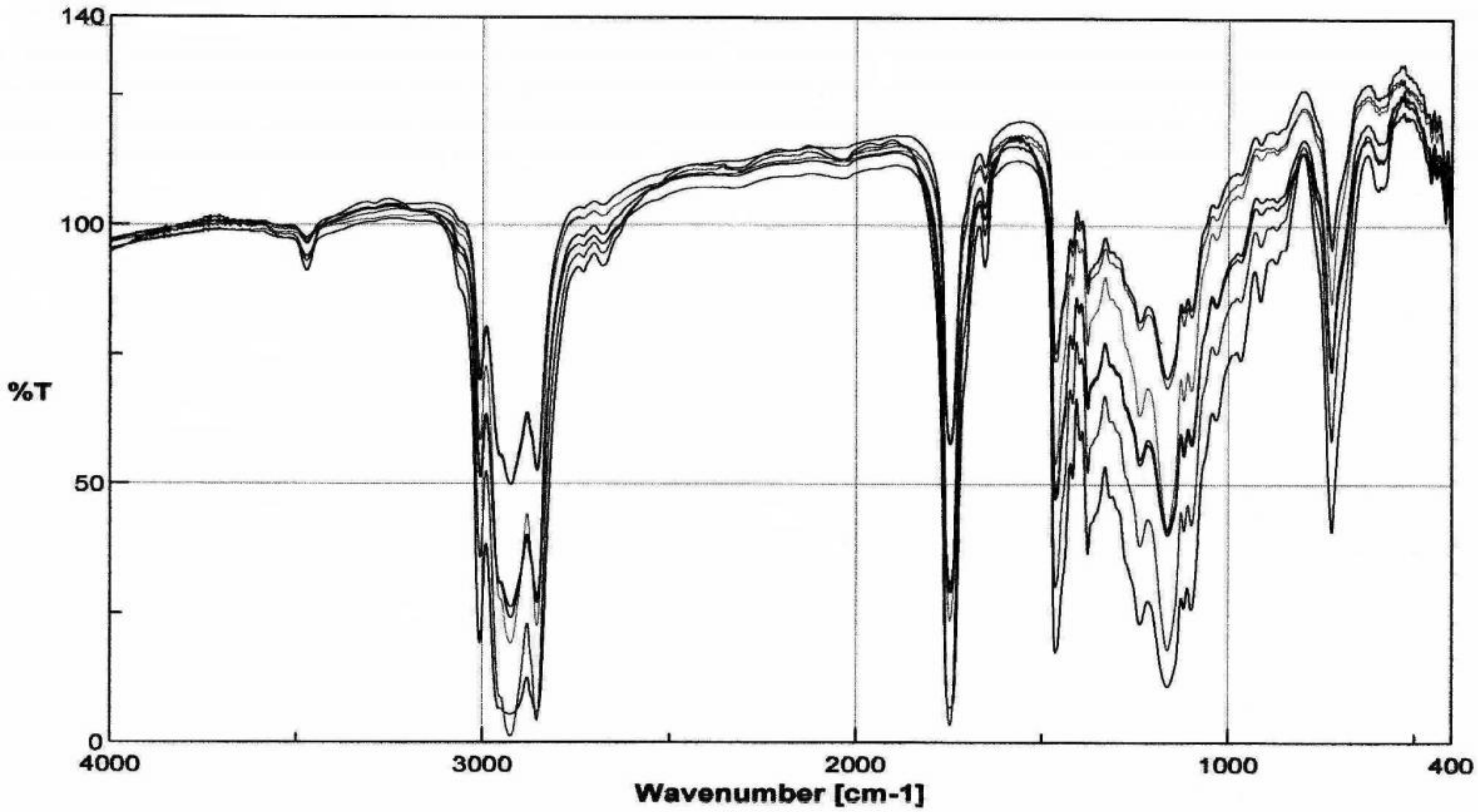

Figure (3) Typical FTIR transmittance spectra of pure extra virgin olive oil (top) mixed with different ratios (0, 5, 10, 20, 30, 40, 50 and downwards till 100%) of corn oil (bottom).

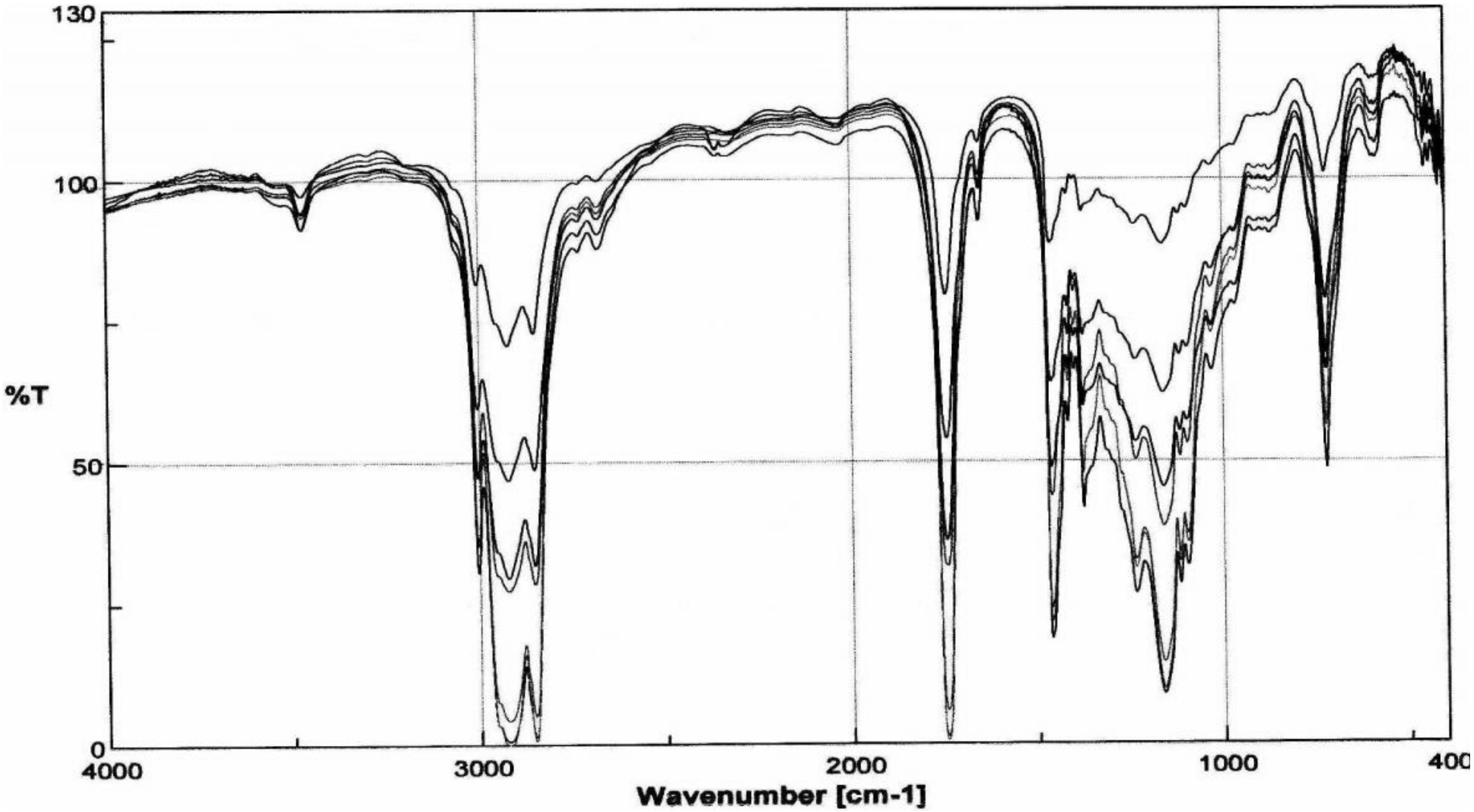

Figure (4) Typical FTIR transmittance Spectra of pure extra virgin olive oil (top) mixed with different ratios (0, 5, 10, 20, 30, 40, 50 and downwards till 100%) of refined olive oil (bottom).

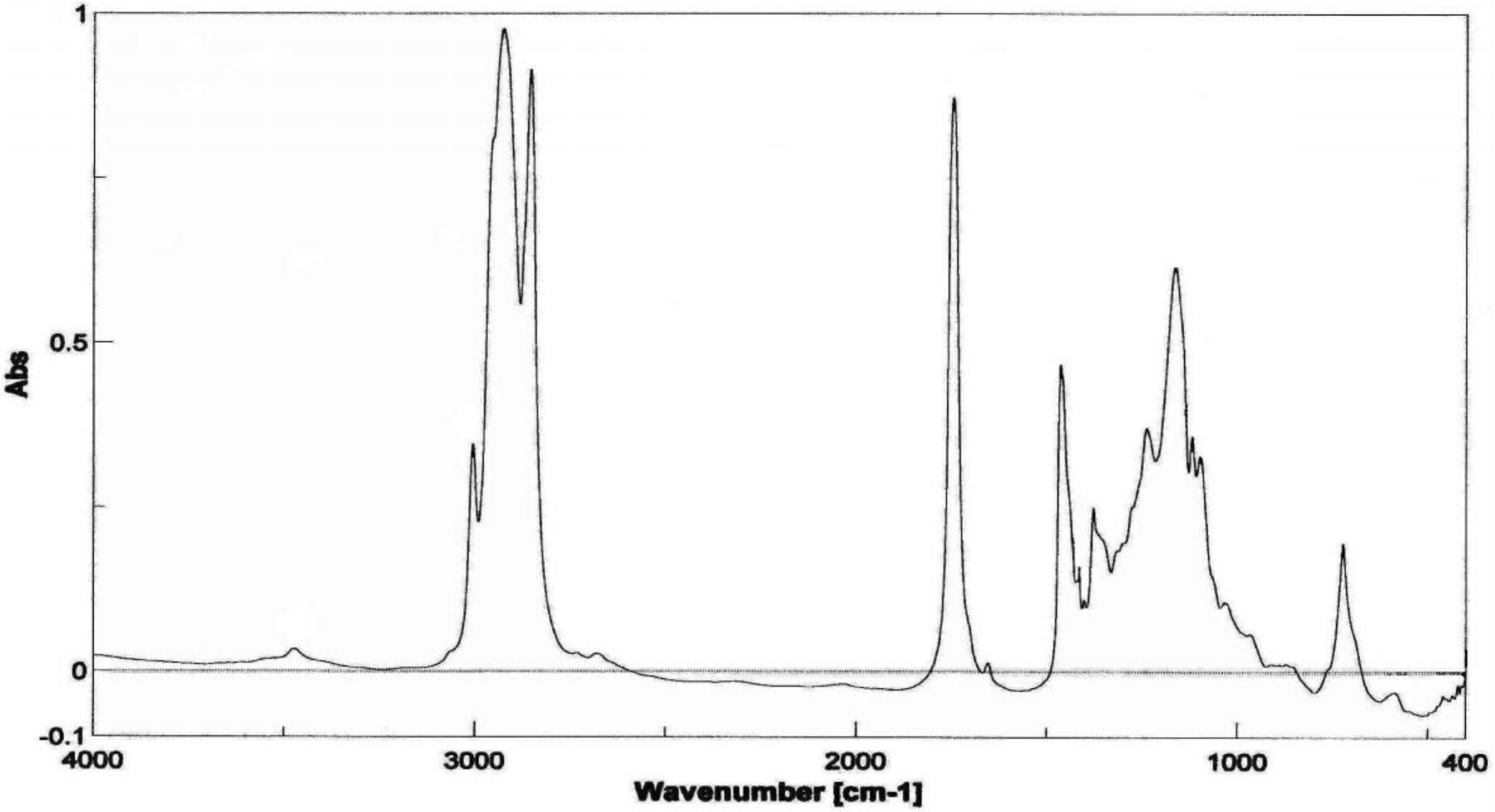

Figure (5) Typical FTIR absorption spectra of extra virgin olive oil

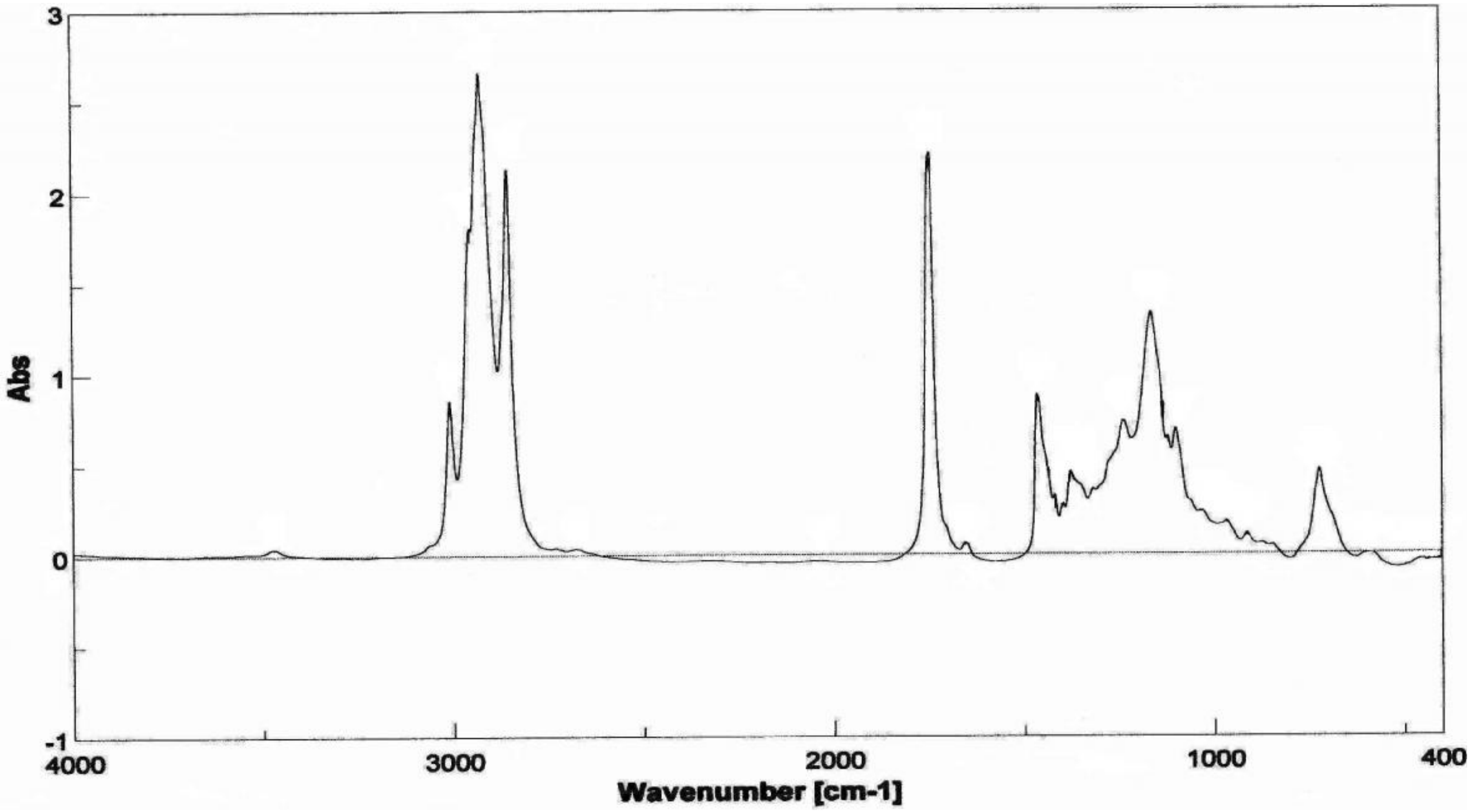

Figure (6) Typical FTIR absorption spectra of 100% sunflower oil

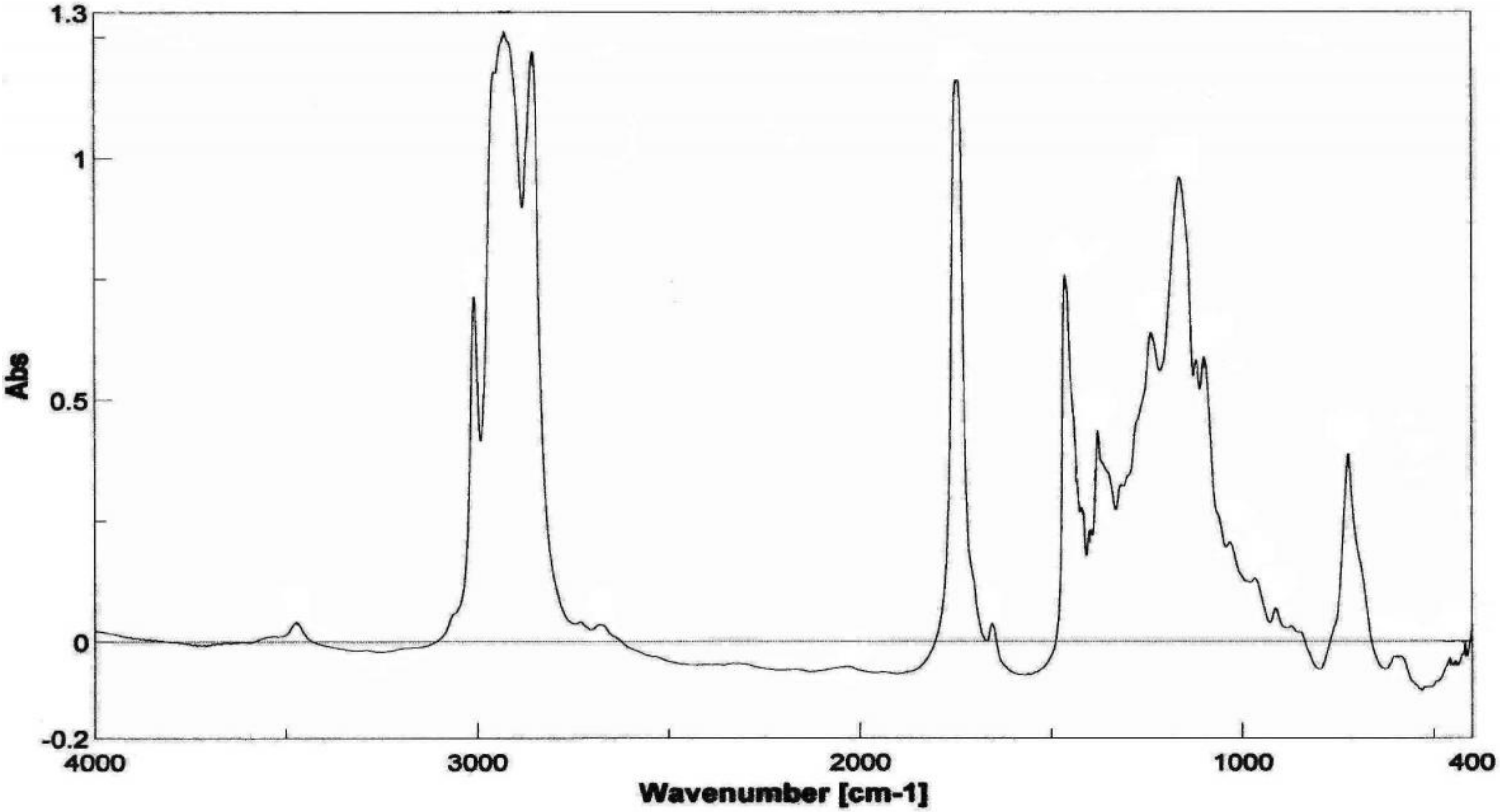

Figure (7) Typical FTIR absorption spectra of 100% corn oil

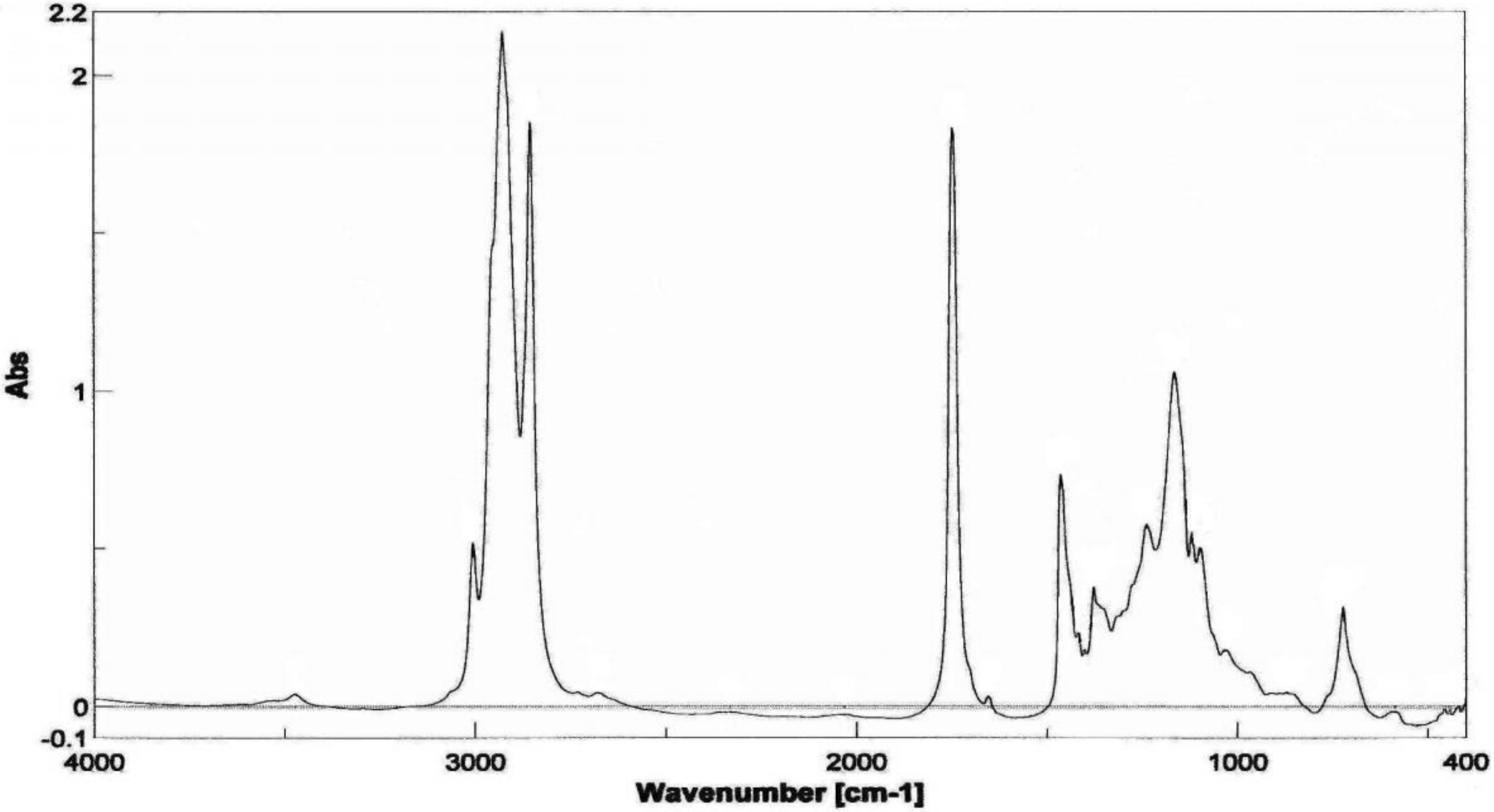

Figure (8) Typical FTIR absorption spectra of 100% refined olive oil

Table (15) Comparison of FTIR spectral data of pure oils (extra virgin olive, sunflower, corn and refined olive oils)

Extra virgin olive oil		**Sunflower oil**		**Corn oil**		**Refined olive oil**	
cm^{-1}	**Abs.%**	**cm^{-1}**	**Abs.%**	**cm^{-1}**	**Abs.%**	**cm^{-1}**	**Abs.%**
3471.24	0.57	3472.2	0.26	3472.2	0.41	3472.2	0.31
3005.52	5.83	3008.41	5.18	3008.41	7.34	3005.52	4.61
		2954.41	10.94				
2925.48	16.40	2926.45	16.14	2926.45	12.94	2926.45	19.10
2854.13	15.38	2854.13	12.92	2854.13	12.54	2854.13	16.54
2679.5	0.45	2678.64	1.33	2679.6	0.36	2679.6	0.39
1747.19	14.67	1747.19	13.31	1748.16	11.93	1747.19	16.40
1653.66	4.03	1654.62	0.39	1654.62	0.37	1654.62	0.30
1463.71	7.84	1463.71	5.32	1463.71	7.76	1463.71	6.53
1417.42	2.62	1418.39	1.98	1418.39	2.98	1418.39	2.17
1402	1.83	1397.17	1.70	1398.14	2.38	1402	1.60
1376.93	4.20	1376.93	2.90	1376.93	4.47	1376.93	3.38
		1319.07	2.16				
1238.08	6.21	1238.08	4.44	1238.08	6.54	1238.08	5.122
1163.83	6.99	1163.83	8.04	1163.83	9.86	1163.83	9.42
1118.51	5.98	1120.44	3.91	1119.48	5.97	1118.51	4.87
1097.30	5.49	1099.23	4.17	1099.23	6.03	1097.3	4.47
1031.73	1.80	1033.66	1.45	1032.69	2.10	1031.73	1.60
		967.126	1.10	967.126	1.34		
912.780	0.24	914.093	0.68	914.093	0.70	914.093	0.39
722.211	3.27	723.175	2.80	723.175	3.98	722.211	2.79

assigned to HC=CH, *cis*, bending out-of-plane. A small peak at 722 cm^{-1} corresponded to CH_2 rocking. These results agree with those reported by **Bertran et al. (1999); Sinelli et al. (2007); Gurdeniz and Ozen (2009).**

Also, it is easily to deduce from Table (15) that the FTIR spectra of sunflower oil were dominated by peaks around 3472, 3008, 2954, 2926, 2854, 2678, 1747, 1645, 1463, 1418, 1397, 1376, 1319, 1238, 1163, 1120, 1099, 1033, 967, 914 and 723 cm^{-1}. Absorbencies at 2926 and 2854 cm^{-1} were due to bands arising from CH_2 stretching vibrations, asymmetric and symmetric, respectively. The major peak at 1747 cm^{-1} aroused from C=O stretching vibrations; the bands at 1463 and 1376 cm^{-1} aroused from CH_2 and CH_3 scissoring vibration, while those bands located at 1238, 1163, 1120 and 1099 cm^{-1} were associated with the C–O stretching vibration. A peak around 967 cm^{-1} was assigned to C–H out-of-plane deformation band, this band is broad with down sloping baseline because of the overlap of the broad absorption of triglycerides and C–H out-of-plane deformation bands arising from the conjugated linoleic acids in the sample and also due to the *trans* isomers in sunflower oil .A peak around 914 cm^{-1} was assigned to HC=CH, (*cis*), bending out-of-plane. A small peak at 723 cm^{-1} was corresponded to CH_2 rocking mode. These results agreed with those reported by **Christy et al. (2003); Sinelli et al. (2007); Gurdeniz and Ozen (2009); Moros et al. (2009); Sherazi et al. (2009).**

Similarly, it is easily to deduce that the FTIR spectra of corn oil are dominated by peaks around 3472, 3008, 2926, 2854, 2679, 1748, 1645, 1463, 1418, 1398, 1376, 1238, 1163, 1119, 1099, 1032, 967, 914 and 723 cm^{-1}. Absorbencies at 2926 and 2854 cm^{-1} were due to bands arising from CH_2 stretching vibrations, asymmetric and symmetric, respectively. The major peak at 1748 cm^{-1} aroused from C=O stretching vibrations; the bands at 1463 and 1376 cm^{-1} aroused from CH_2 and CH_3 scissoring vibration, while those bands located at 1238, 1163, 1119 and 1099 cm^{-1} were associated with C–O stretching vibration. A peak around 967 cm^{-1} was assigned to C–H out-of-plane deformation band, this band was broad with down sloping baseline because of the overlap of the broad absorption of triglycerides and C–H out-of-plane deformation bands arising from the conjugated linoleic acids in the sample and also due to the *trans* isomers in corn oil. A peak around 914 cm^{-1} was assigned to HC=CH, (*cis*), bending out-of-plane. A small peak at 723 cm^{-1} was corresponded to CH_2 rocking. These results agree with those reported by **Christy et al. (2003); Sinelli et al. (2007); Gurdeniz and Ozen (2009); Sherazi et al. (2009).**

Last, it is easily to deduce that the FTIR spectra of refined olive oil were dominated by peaks around 3472, 3005, 2926, 2854, 2679, 1747, 1645, 1463, 1418, 1402, 1376, 1238, 1163, 1118, 1097, 1031, 914 and 722 cm^{-1}. Absorbencies at 2926 and 2854 cm^{-1} were due to bands arising from CH_2 stretching vibrations, asymmetric and symmetric, respectively. The major peak at 1747 cm^{-1} aroused from C=O stretching vibrations, the bands at 1463 and 1376 cm^{-1} aroused from CH_2 and CH_3 scissoring vibration, while those bands located at 1238, 1163, 1118 and 1097 cm^{-1} were associated with C–O stretching vibration. A peak around 914 cm^{-1} was assigned to HC=CH, (*cis*), bending out-of-plane. A small peak at 722 cm^{-1} was corresponded to CH_2 rocking mode. These results agreed with those reported by **Sinelli et al. (2007); Gurdeniz and Ozen (2009).**

As shown above, one can draw a conclusion that all the pure oils and blends used in this investigation showed many similarities between their FTIR spectral data concerning wavenumbers or intensities or shapes of peaks which make the differentiation between them very difficult. However, when we closely examine the FTIR spectral data we shall find some variations concerning some wavenumbers and the intensity of absorption if compared at the same specified wavenumber. These variations successfully led to the detection of adulteration of the extra virgin olive oil with other adulterant oils even at low concentrations of the adulterant oils. There were some individual peaks with characteristic wavenumber which was identical with their corresponding oil. These characteristic peaks include the absorption peaks of extra virgin olive oil and refined olive oil at 3005 and 1402 cm^{-1}, the absorption peaks of sunflower oil at 2954, 1397, 1319 and 967 cm^{-1} and the absorption peaks of corn oil at 3008, 1398 and 967 cm^{-1}. Also, there were characteristic peaks of higher absorption intensities at the same wavenumbers and there exists some wavenumber shifts for different oils.

In all the non-oxidized oil infrared spectra, a band near 3471 cm^{-1} was associated with the overtone of the glyceride ester carbonyl absorption. As the oxidation process advances the concentration of hydroperoxide groups in the sample increases and also its absorption in the infrared spectrum. This functional group gives a broad band, so it overlaps with that of the overtone of the glyceride ester groups, producing a decreasing in the frequency value of the glyceride original band together with an increasing in its absorbance. For this reason, frequency and absorbance of this band can give information about the hydroperoxide generation throughout the oxidation process. In FTIR spectrum near 3005 cm^{-1} appears the band of *cis*- double bonds (assigned to =C–H groups). In non-oxidized oil samples it has been shown that the frequency of this band is related to the composition of the oil; oils with a large proportion of polyunsaturated acyl groups have higher frequency values (such as 3008 cm^{-1}) than those with a large proportion of monounsaturated or saturated acyl groups. During the oxidation process, the disappearance of *cis*- double bonds is produced, as well as isomerization of *cis*- to *trans*-groups, alongside with hydroperoxide generation. For this reason frequency and absorbance of band near 3005 cm^{-1} suffer changes as the oxidation process advances. It can be observed that the time at which the frequency of this band begins to decrease is also a measurement of the oil stability. These results agree with those reported by **Russin et al. (2003).**

There are other bands, near 1238, 1163 and 1118 cm^{-1}, related to the stretching vibrations of the C–O groups of esters, whose frequency values show an evolution similar to that observed in band near 3005 cm^{-1}, throughout the oxidation process. The non-oxidized oil samples show the band of the triglyceride ester groups at 1747 cm^{-1}. During the oxidation process hydroperoxides degrade into secondary oxidation products such as aldehydes and ketones, which give bands near 1728 cm^{-1}. These bands overlap with that of ester group at 1747 cm^{-1} causing a broadening of the band and a decreasing of its frequency. Although peroxide value, anisidine value and other classic parameters are used in the measurement of oil oxidative stability, infrared data can be used for the same task with many advantages: the infrared spectrum can give information on the different functional groups present in the sample in significant proportions; the amount of oil sample needed to collect a spectrum is very small; preparation of the sample and collection of the spectrum only takes a couple of minutes; error sources in the sample preparation, spectrum collection, and frequency and absorbance determination are minimal because these latter are automatic and because the sample preparation does not require any manipulation; finally, the cost is also minimal because no reactive is needed. For these reasons infrared data may replace the classic parameters not only in routine controls but also in research on oxidative stability or on antioxidant activity **(Guillén and Cabo, 2002).**

4.4.3- Detection of adulteration of extra virgin olive oil with adulterant sunflower oil using FTIR spectral data analysis:

As shown in Figures (9, 10, 11, 12, 13 and 14), these 6 figures represent FTIR spectral data analysis of the binary admixtures of extra virgin olive oil with adulterant sunflower oil at the gradual increasing concentrations of 5, 10, 20, 30, 40 and 50%. As formerly noticed from Figure (6) representing pure sunflower oil (concentration of sunflower oil is 100%), a band shift observed at 3008 cm^{-1} assigned to the C–H stretching vibration of the *cis*-double bond (=C–H) has primarily showed absorbance at 3005 cm^{-1} in case of extra virgin olive oil represented in Figure (5).

When examining the FTIR spectral data analysis of sunflower oil beginning from its concentration of 5% in Figure (9) till 50% in Figure (14), it was noticed an absorbance at 3005 cm^{-1} corresponding to 5% sunflower oil and 10% sunflower oil; absorbance at 3006 cm^{-1} corresponding to 20% and 30% sunflower oil; absorbance at 3007 cm^{-1}

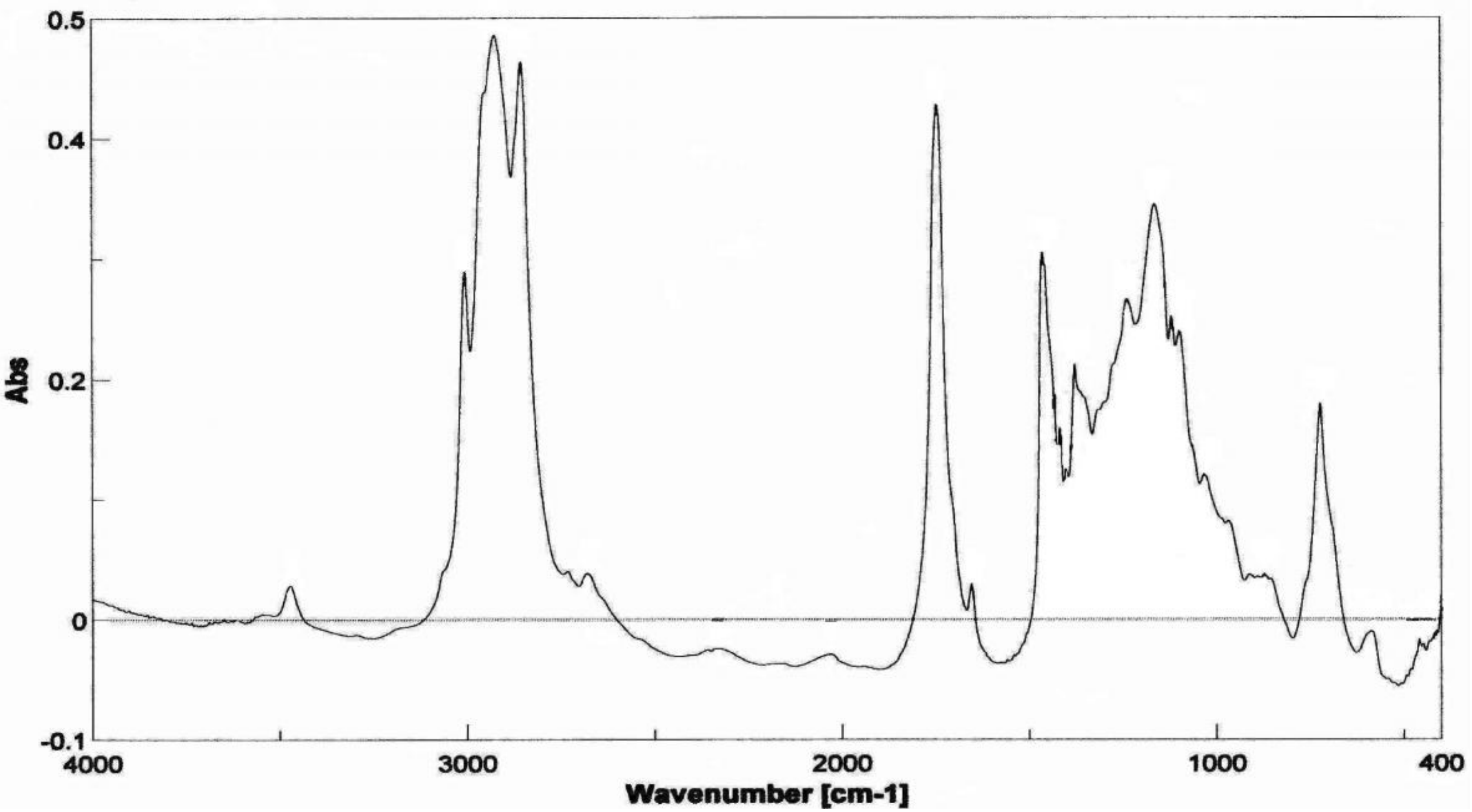

Figure (9) Typical FTIR absorption spectra of 5% sunflower oil

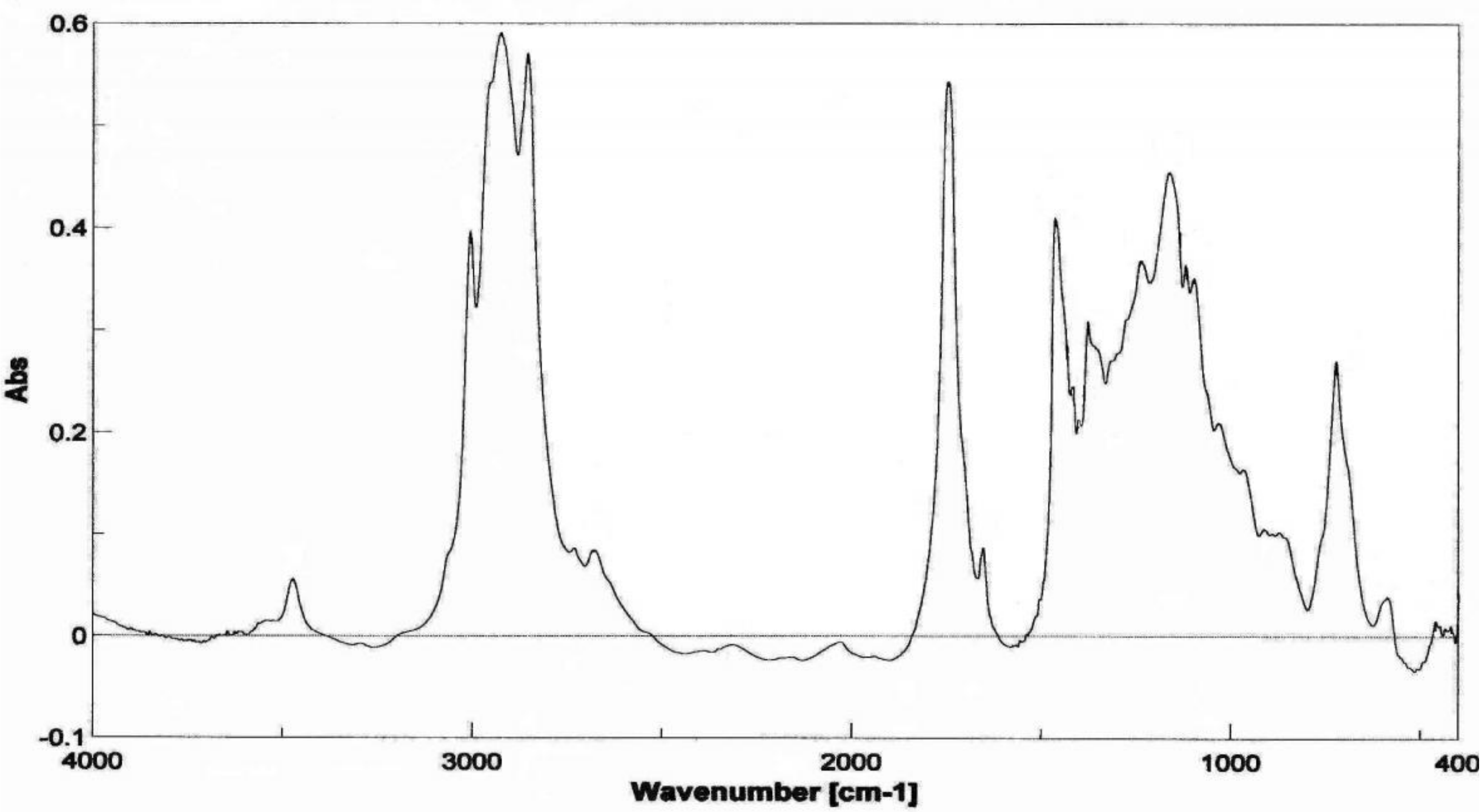

Figure (10) Typical FTIR absorption spectra of 10% sunflower oil

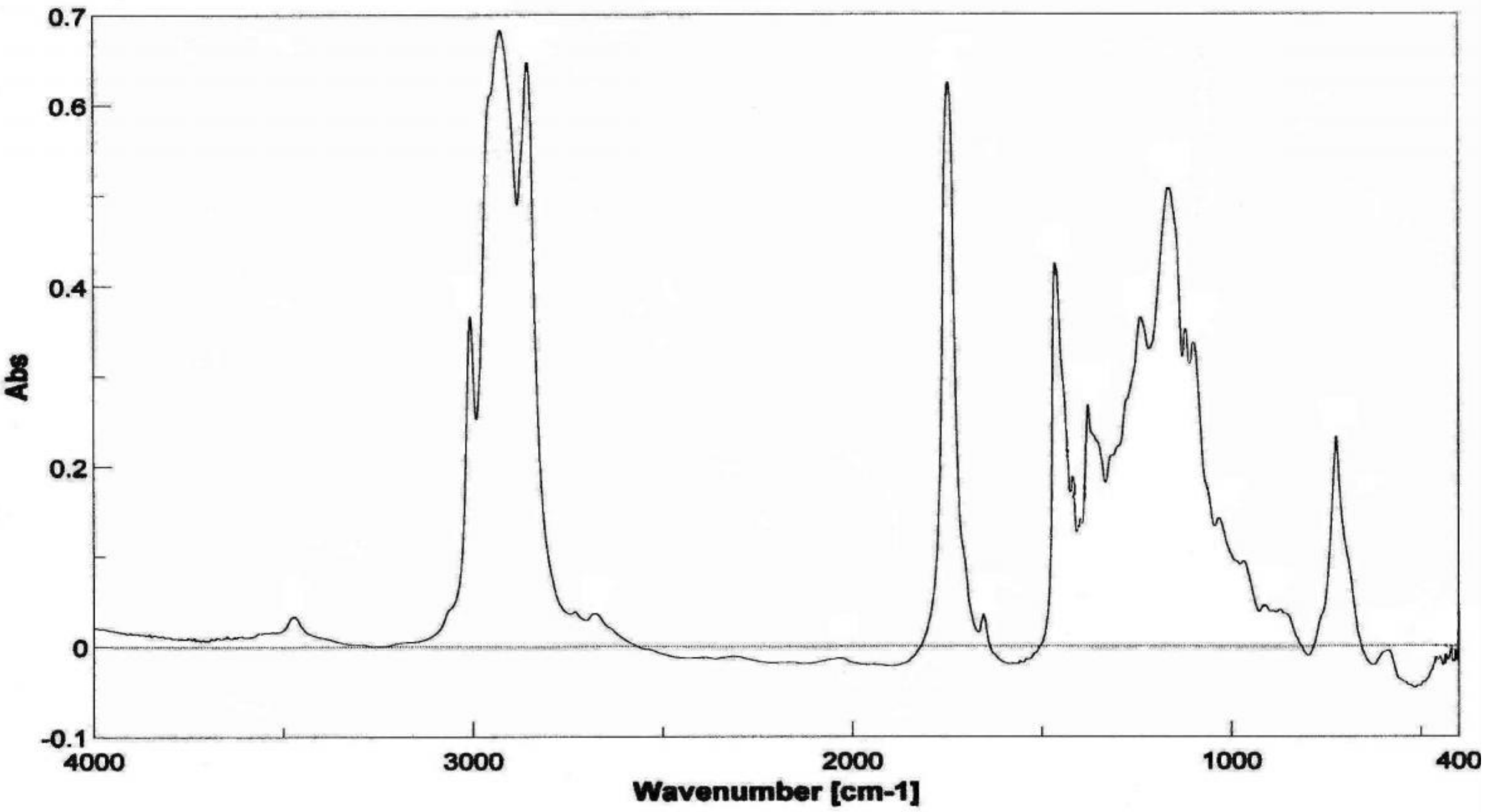

Figure (11) Typical FTIR absorption spectra of 20% sunflower oil

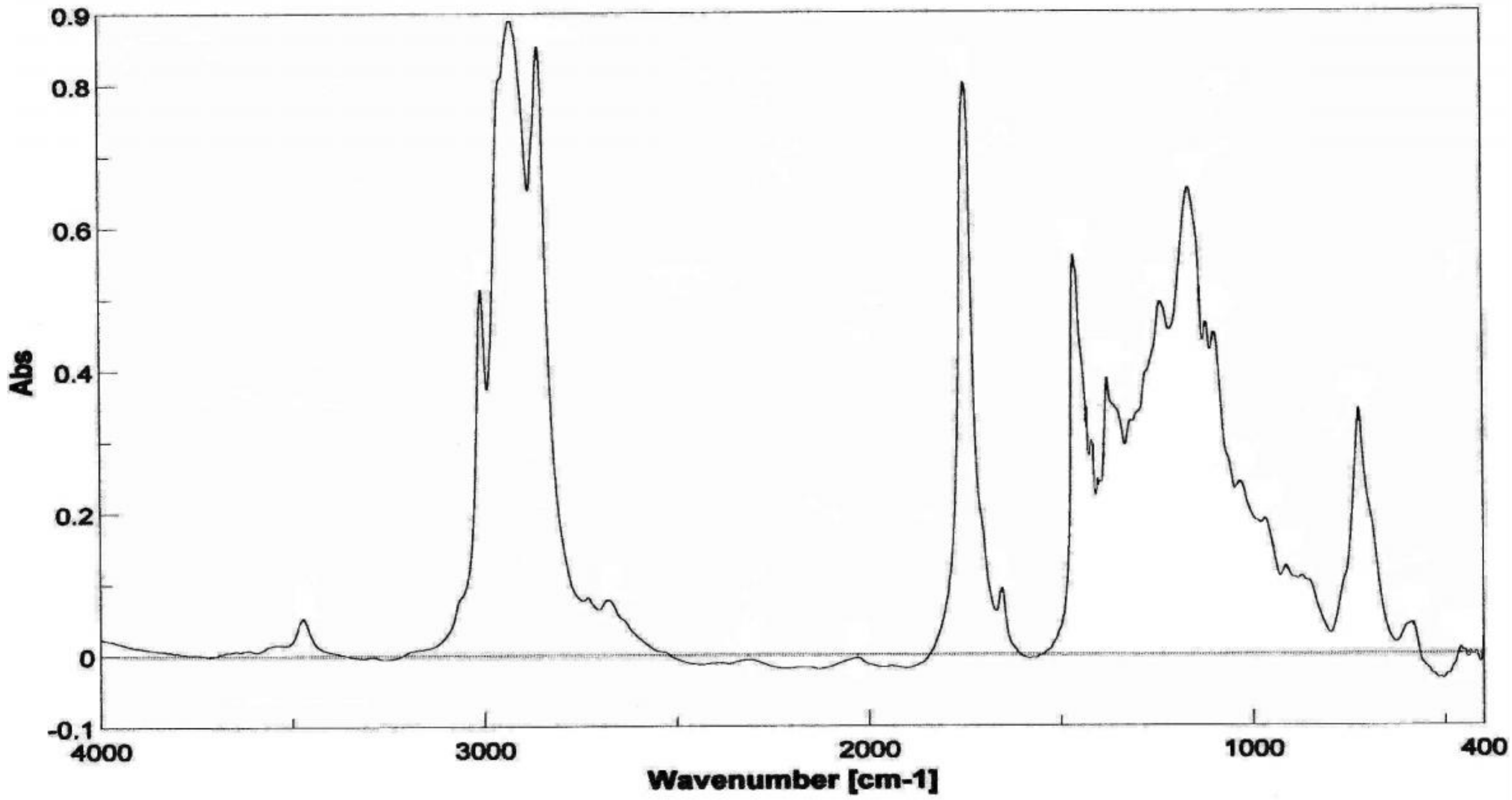

Figure (12) Typical FTIR absorption spectra of 30% sunflower oil

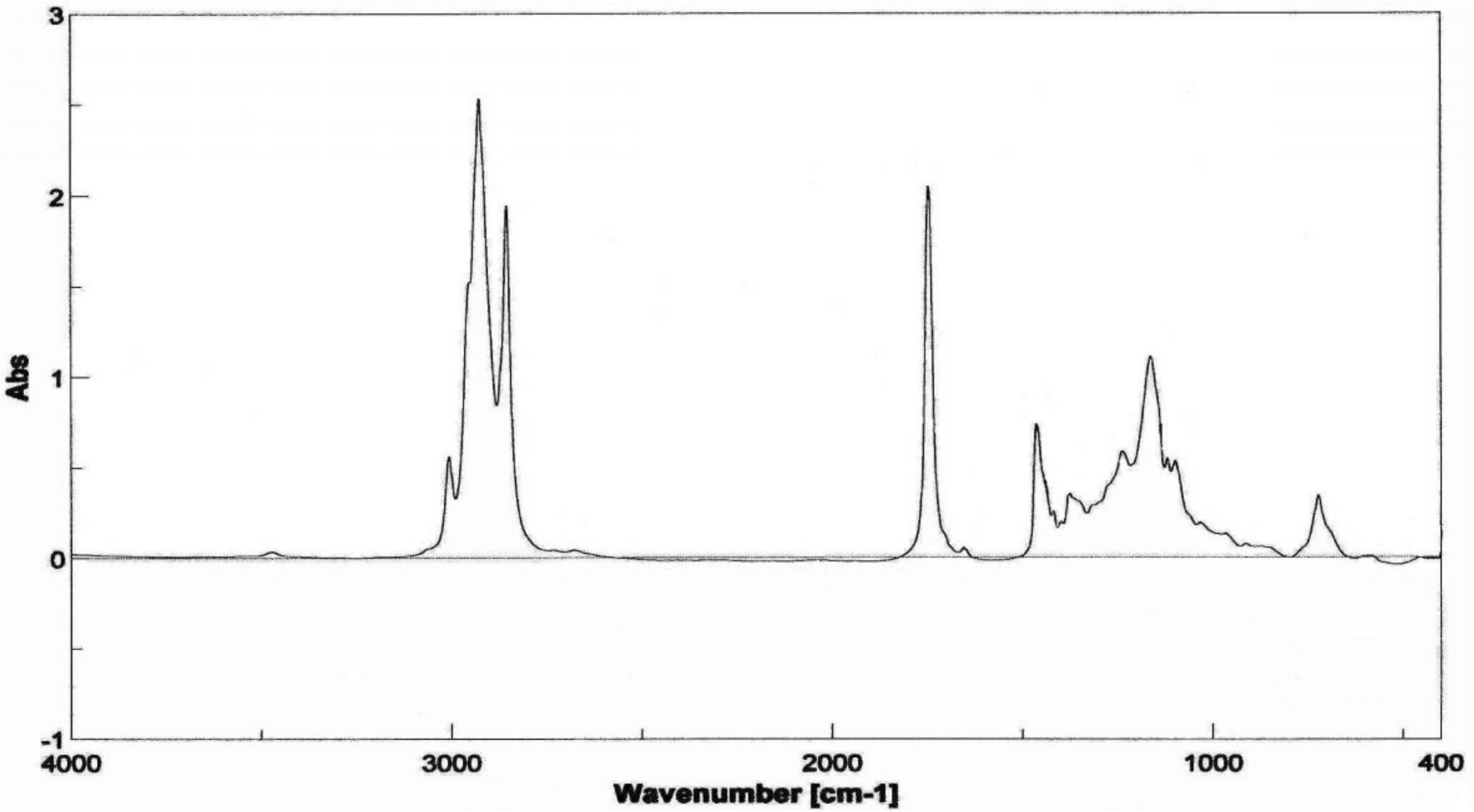

Figure (13) Typical FTIR absorption spectra of 40% sunflower oil

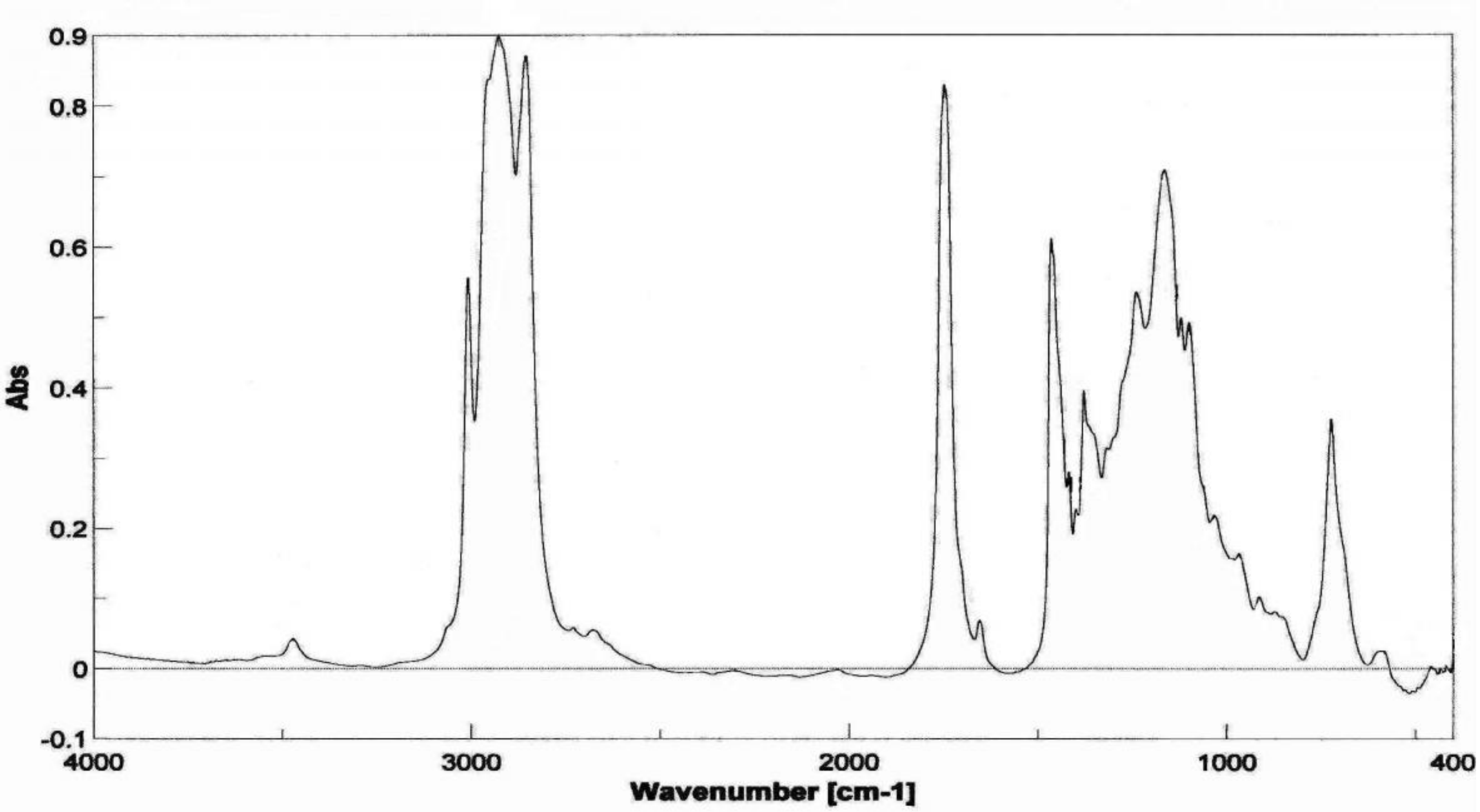

Figure (14) Typical FTIR absorption spectra of 50% sunflower oil

corresponding to 40% and 50% sunflower oil in the blend. These observations indicated that the band shifted away from 3005 cm^{-1} (characteristic of extra virgin olive oil) starting from the concentration of 20% of the adulterant sunflower oil which gave absorption at 3006 cm^{-1}, but the concentration of 10% sunflower oil gave absorption at 3005 cm^{-1} which is similar to extra virgin olive oil. With gradual increasing of adulterant sunflower oil concentration from 20% up to 100%, it was found an increase of the band shifted away from 3005 cm^{-1}, till it reaches its maximum shift at 100% sunflower oil with absorbance at 3008 cm^{-1}. These findings allow the detection of extra virgin olive oil adulteration with sunflower oil beginning from its concentration of 20% in the blend.

It is clear that the oil composition affects the exact position of the band and yields shifts when the proportion of the fatty acid changes. In this way, the value of this frequency in non-oxidized oil samples varies significantly from 3008 to 3005 cm^{-1}. For example, sunflower oil, soybean oil, corn oil and sesame seed oil show a maximum absorbance at 3008 cm^{-1} comparing to extra virgin olive oil that has a maximum of absorbance at 3005 cm^{-1}. These are due to their composition, as vegetable oils contain certain higher proportion of linolenic or linoleic acyl groups, whereas extra virgin olive oil contains higher proportion of oleic acyl groups. Moreover, as the sunflower oil is gradually mixed in various proportions with extra virgin olive oil, the clear shift of the 3005 cm^{-1} band, attributed to the C–H stretching vibration of *cis*-double bond, to the 3008 cm^{-1} band which is noticed.

Also, it was observed a shift of the absorption band at 2926 cm^{-1}. This band is attributed to the symmetric stretching vibration of the aliphatic CH_2 group which has primarily showed absorbance at 2925 cm^{-1} in case of extra virgin olive oil represented in Figure (5). This band shifted from 2925 to 2926 cm^{-1} beginning from the concentration of 20% of adulterant sunflower oil. In order to quantify further the adulteration, the maximum heights of the two bands at 3005 and 2926 cm^{-1} were also used. The height of the 3005 cm^{-1} band for the extra virgin olive oil sample is obviously smaller than it is for all the types of vegetable oils, and changes according to the extent of adulteration. When the percentage of added vegetable oil increases, the height of this band also increases, approaching the height that matches to the vegetable oil, when the adulteration is quite high.

The 2925 cm^{-1} band height exhibits small changes and not in a specific way, as the 3005 cm^{-1} band. Also, a peak of considerable intensity was observed at 2954 cm^{-1} in pure sunflower oil only as in Table (15). This absorption peak was attributed to symmetric stretching vibration shoulder of the aliphatic CH_3 group. This peak appeared as a small shoulder lied on the left hand side of the major absorption peak at 2926 cm^{-1} arising from symmetric stretching vibration of the aliphatic CH_2 group. The relationship between shift of absorption peak and concentrations of adulterant sunflower oil was tabulated in Table (20) and plotted in Figure (31) which showed a linear relationship. These results harmonized with those reported by **Vlachos et al. (2006).**

Also, a band shift was observed at 914 cm^{-1} concerning the pure sunflower oil which was 912 cm^{-1} in case of extra virgin olive oil. With gradual addition of adulterant sunflower oil, the value of band was shifted away from 912 cm^{-1} (characteristic of extra virgin olive oil). We attained absorbance at 912 cm^{-1} corresponding to 5% sunflower oil; absorbance at 913 cm^{-1} corresponding to 10, 20, 30 and 40% sunflower oil in the blends and absorbance at 914 cm^{-1} corresponding to 50% sunflower oil. This findings indicated that the band was shifted away from 912 cm^{-1} (characteristic of extra virgin olive oil) starting from the concentration of 10% of adulterant sunflower oil which gave absorption at 913 cm^{-1}, while the concentration of 5% sunflower oil gave absorption at 912 cm^{-1} which is the same of extra virgin olive oil.

Concerning the absorption peak at 1163 cm^{-1}, it was noticed that there was some increases in the absorption intensity values with the gradual addition of adulterant sunflower oil beginning from the 5% concentration till pure sunflower oil. This relationship was tabulated in

Table (18) and plotted in Figure (29). There exists an important absorption peak of considerable intensity at 1163 cm^{-1} due to C–O stretching and C–H bending. There was no shifts away from this wavenumber in FTIR spectral data, but there was some variations in absorption intensities with varying the concentration of the adulterant sunflower oil in such a way to be valuable to be tabulated in Table (18) with its related plot in Figure (29) which shows the relationship between changes in absorption intensity values at 1163 cm^{-1} and the concentrations of adulterant sunflower oil. It is shown that the absorption intensity of extra virgin olive oil was evaluated as 0.416. With gradual addition of adulterant sunflower oil concentrations, the absorption intensity values were 0.445, 0.455, 0.509, 0.653, 0.708, 0.907 and 1.329 for the concentrations of 5%, 10%, 20%, 30%, 40%, 50% and 100% sunflower oil, respectively. This means that it is possible to detect the adulteration of extra virgin olive oil with adulterant sunflower oil beginning from 5% sunflower oil in the blend using the absorption intensity at 1163 cm^{-1}. These results are in excellent agreement with those reported by **Allam and Hamed (2007).**

Also, there was an important absorption band at 967 cm^{-1} which was found in FTIR spectra of sunflower oil and was absent in FTIR spectra of extra virgin olive oil. This wavenumber along with some absorption intensity was found only when the concentrations of adulterant sunflower oil was 30% and above in the blend till pure sunflower oil. The absorption peak at 967 cm^{-1} was due to C–H out-of-plane deformation band. This band is broad with down sloping baseline because of the overlap of the broad absorption of triglycerides and of C–H out-of-plane deformation bands arising from the conjugated linoleic acids (CLA) in the sample. Also, the isolated *trans*- isomers in oils and fats give rise to a weak absorption around 967 cm^{-1}. The problems encountered in the determination of *trans*- content in fats and oils by infrared spectrometry using the absorption at 967 cm^{-1} lie in the fact that there was no ideal reference material that can remove the sloping of the *trans*- band at 967

cm^{-1}. This is because the triglycerides of fats and oils contain a variety of combinations of fatty acids in the molecules and the absorptions in the infrared spectrum arising from the fatty acid groups are affected by the other fatty acids present in a glyceride molecule.

Researchers have been trying to find remedies to improve the ways to quantify the absorption at 967 cm^{-1} by experimenting modifications both in the sample preparation and in the ways of quantification because of the importance of conjugated linoleic acids (CLA) which are a mixture of geometrical and positional isomers of linoleic acid with conjugated double bonds. These isomers can also be found in partially hydrogenated corn and sunflower oils that are rich in linoleic acids. Also, it was found that the peak at 967 cm^{-1} found in FTIR spectra of mixtures of sunflower oil with extra virgin olive oil with adulterant sunflower oil of 30% and above, was due to C=C bending mode of molecule; *trans*- RHC=CHR. These results correspond to those reported by **Yang and Irudayaraj (2001); Christy et al. (2003).**

Also, if we examine again the FTIR spectra of extra virgin olive oil and sunflower oil, we will find an important peak ascribed to the very weak stretching of the C=O of the acid group located at 1653 cm^{-1} with considerable absorption intensity and this peak appears as a shoulder of the very strong band of the C=O stretching band of the ester group located at 1747 cm^{-1}. This peak is considered as an important tool because it represents the acidity of the extra virgin olive oil. When comparing the absorption intensities at 1653 cm^{-1} for extra virgin olive oil alone and with its mixtures with sunflower oil, we found that it has higher absorption in case of extra virgin olive oil compared to its mixtures with sunflower oil which interpret the higher acidity of extra virgin olive oil, and this results came in accordance with the results obtained from the manual chemical analysis which showed the higher acidity of extra virgin olive oil and the lower acidity obtained from the mixtures of extra virgin olive oil with sunflower oil at any concentrations of adulterant sunflower oil. These observations conform to those reported by **Iñón et al. (2003).**

4.4.4- Detection of adulteration of extra virgin olive oil with adulterant corn oil using FTIR spectral data analysis:

As shown in Figures (15, 16, 17, 18, 19 and 20), these 6 figures represent FTIR spectral data analysis of the extra virgin olive oil samples in binary admixtures with gradual increasing of corn oil as adulterant one at different concentrations of 5, 10, 20, 30, 40 and 50%. As formerly noticed from Figure (7) representing pure corn oil, a band shift observed at 3008 cm^{-1} assigned to the C–H stretching vibration of the *cis*- double bond (=C–H) which has primarily showed absorbance at 3005 cm^{-1} in case of extra virgin olive oil represented in Figure (5). When running after the FTIR spectral data analysis of corn oil beginning from its concentration of 5% in Figure (15) till 50% concentration in Figure (20), we light upon absorbance at 3005 cm^{-1} corresponding to 5% and 10% corn oil; absorbance at 3006 cm^{-1} corresponding to 20% and 30% corn oil; absorbance at 3007 cm^{-1} corresponding to 40% and 50% corn oil. This indicates that the band shifted away from 3005 cm^{-1} (as a characteristic of extra virgin olive oil) starting from the concentration of 20% of adulterant corn oil which gave absorption at 3006 cm^{-1}, meanwhile the 10% corn oil gave an absorption at 3005 cm^{-1} which was the same of extra virgin olive oil. With gradual increasing of adulterant corn oil concentration from 20% up to 100%, it was found an increase of the band shifted away from 3005 cm^{-1}, till it reached its maximum shift at 100% corn oil with absorbance at 3008 cm^{-1}. These findings allow the detection of extra virgin olive oil adulteration with corn oil beginning from 20% adulterant corn oil.

It is clear that the oil composition affects the exact position of the band and yields shifts when the proportion of the fatty acid changes. In this way, the value of this frequency in non-oxidized oil samples varies significantly from 3008 to 3005 cm^{-1}. For example, corn oil, sunflower oil, soybean oil and sesame seed oil showed a maximum absorbance at 3008 cm^{-1} comparing to extra virgin olive oil that has a maximum of absorbance at 3005 cm^{-1}.

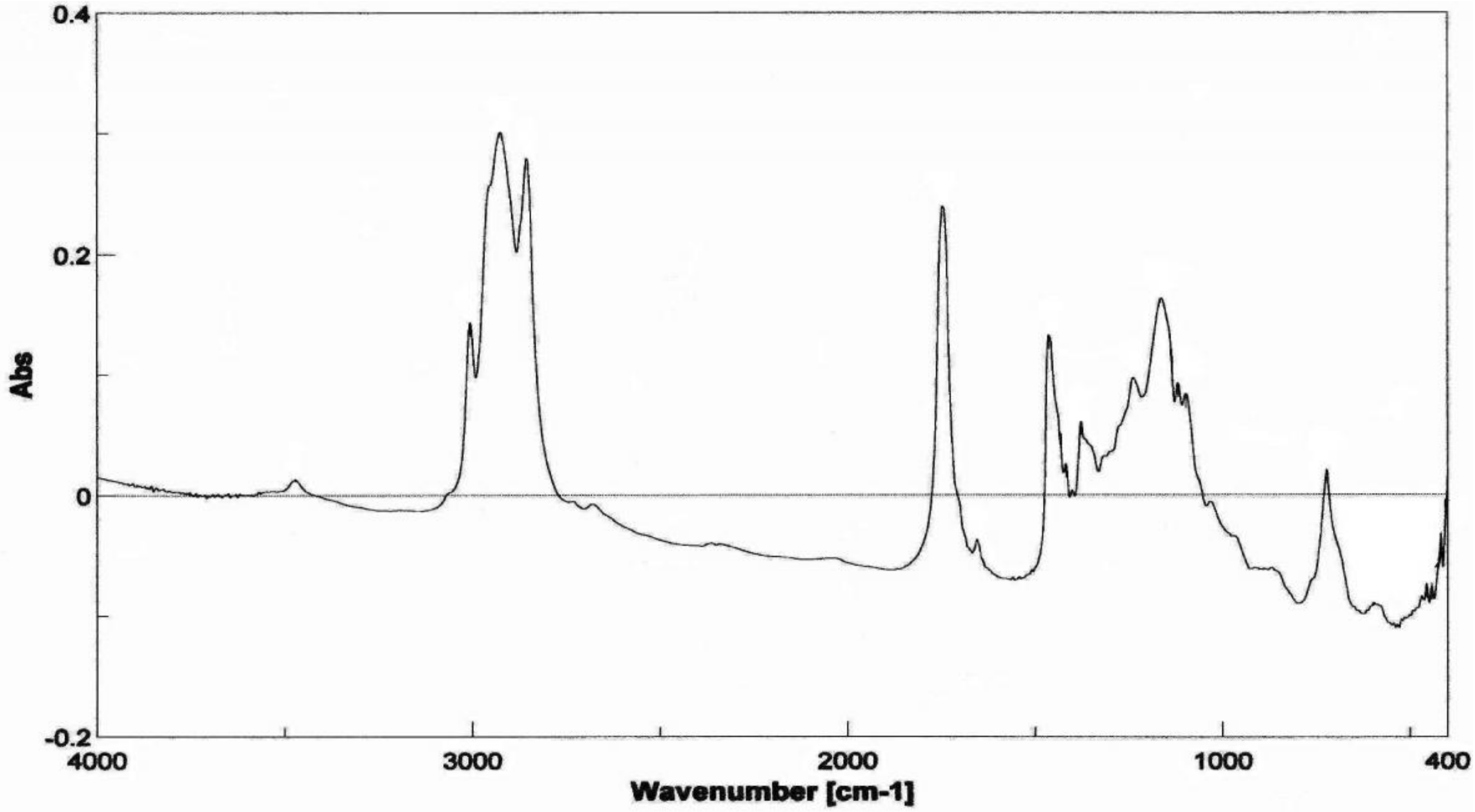

Figure (15) Typical FTIR absorption spectra of 5% corn oil

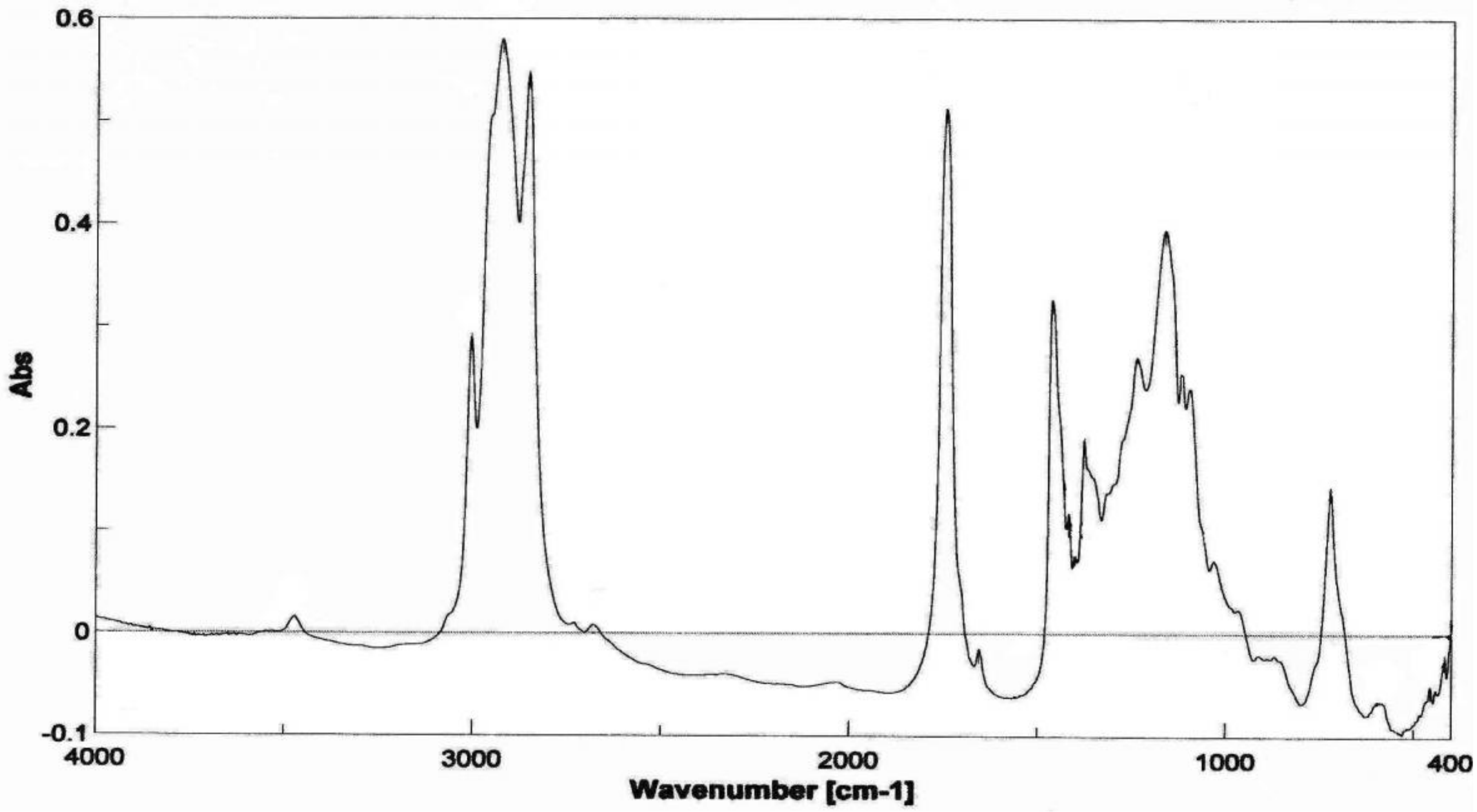

Figure (16) Typical FTIR absorption spectra of 10% corn oil

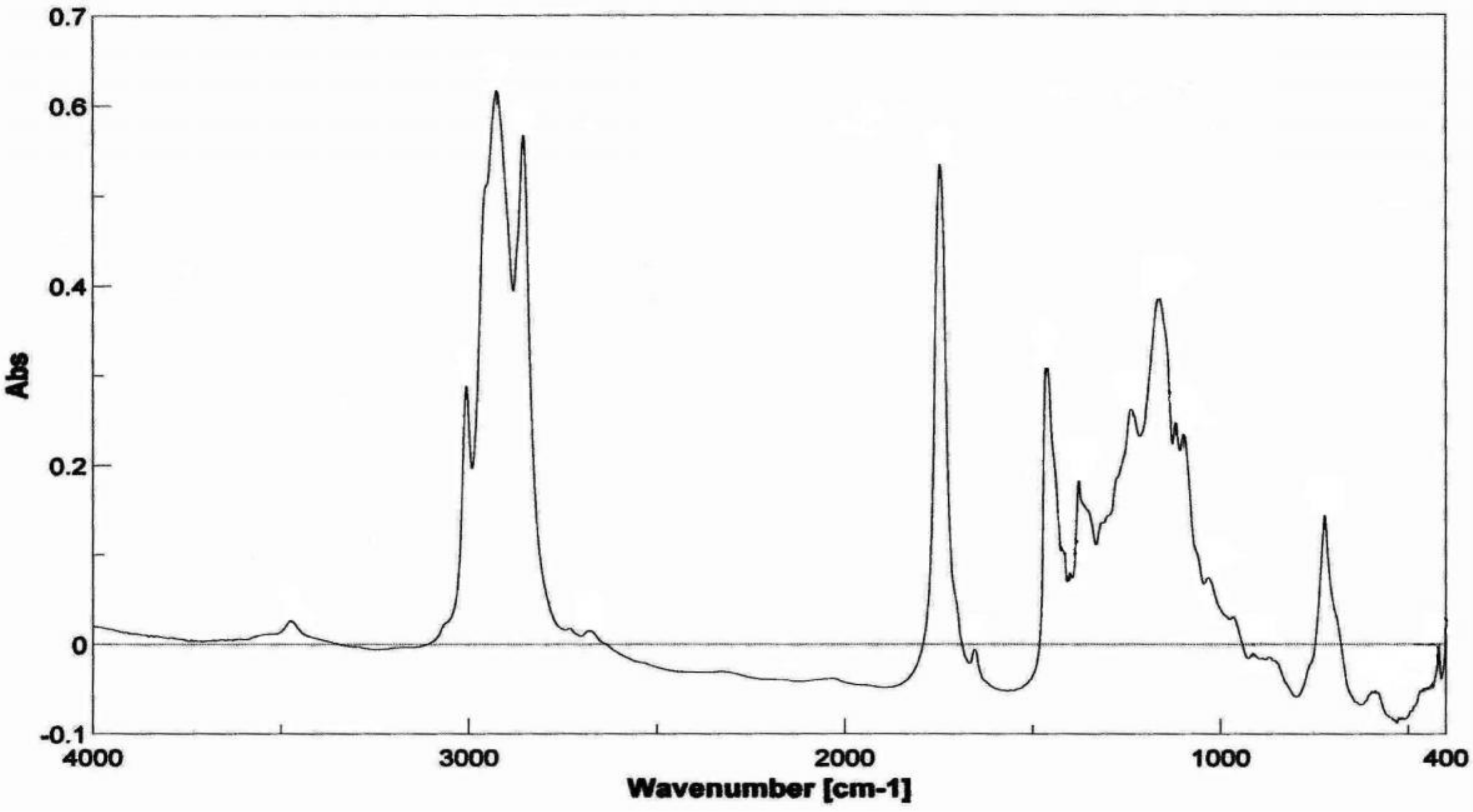

Figure (17) Typical FTIR absorption spectra of 20% corn oil

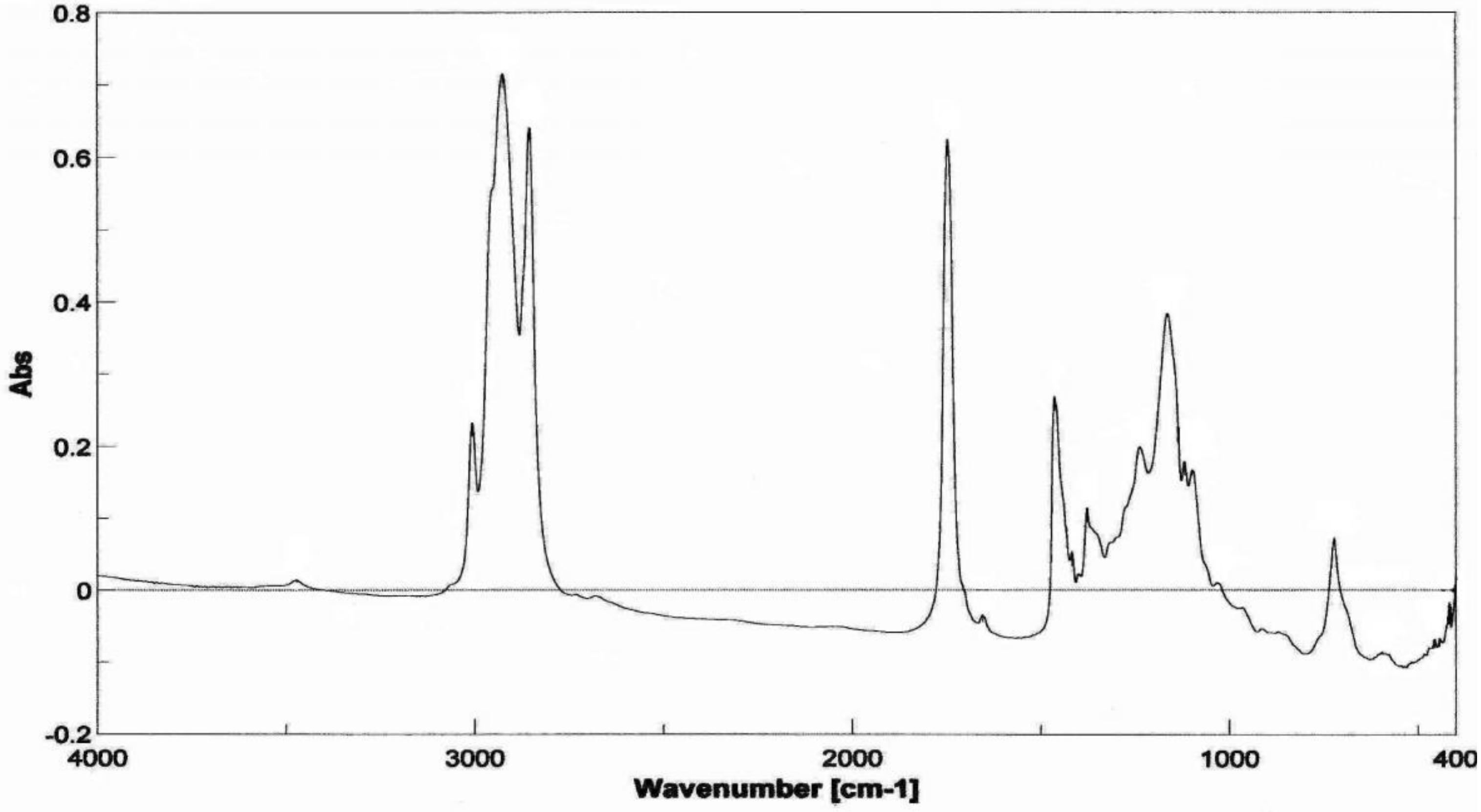

Figure (18) Typical FTIR absorption spectra of 30% corn oil

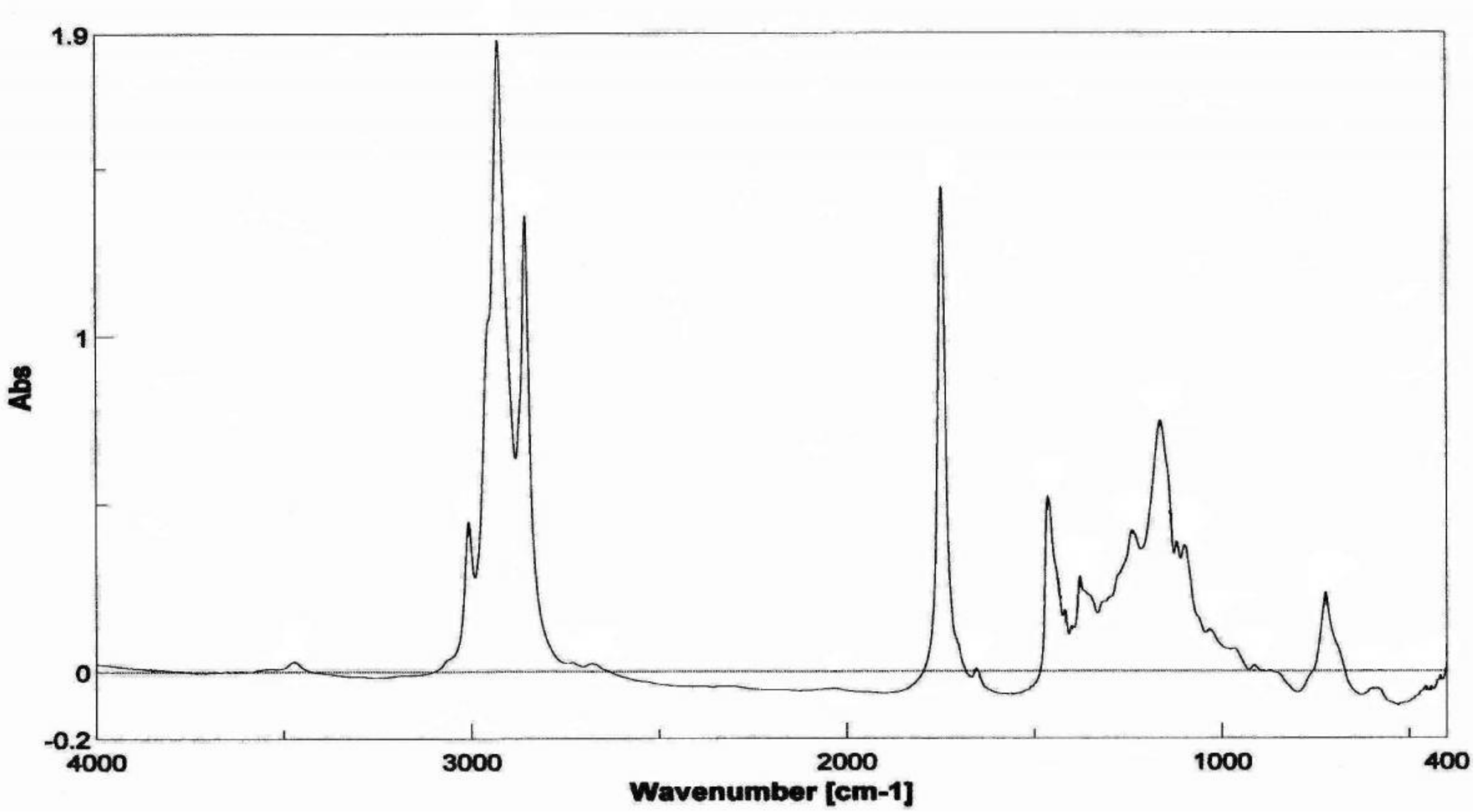

Figure (19) Typical FTIR absorption spectra of 40% corn oil

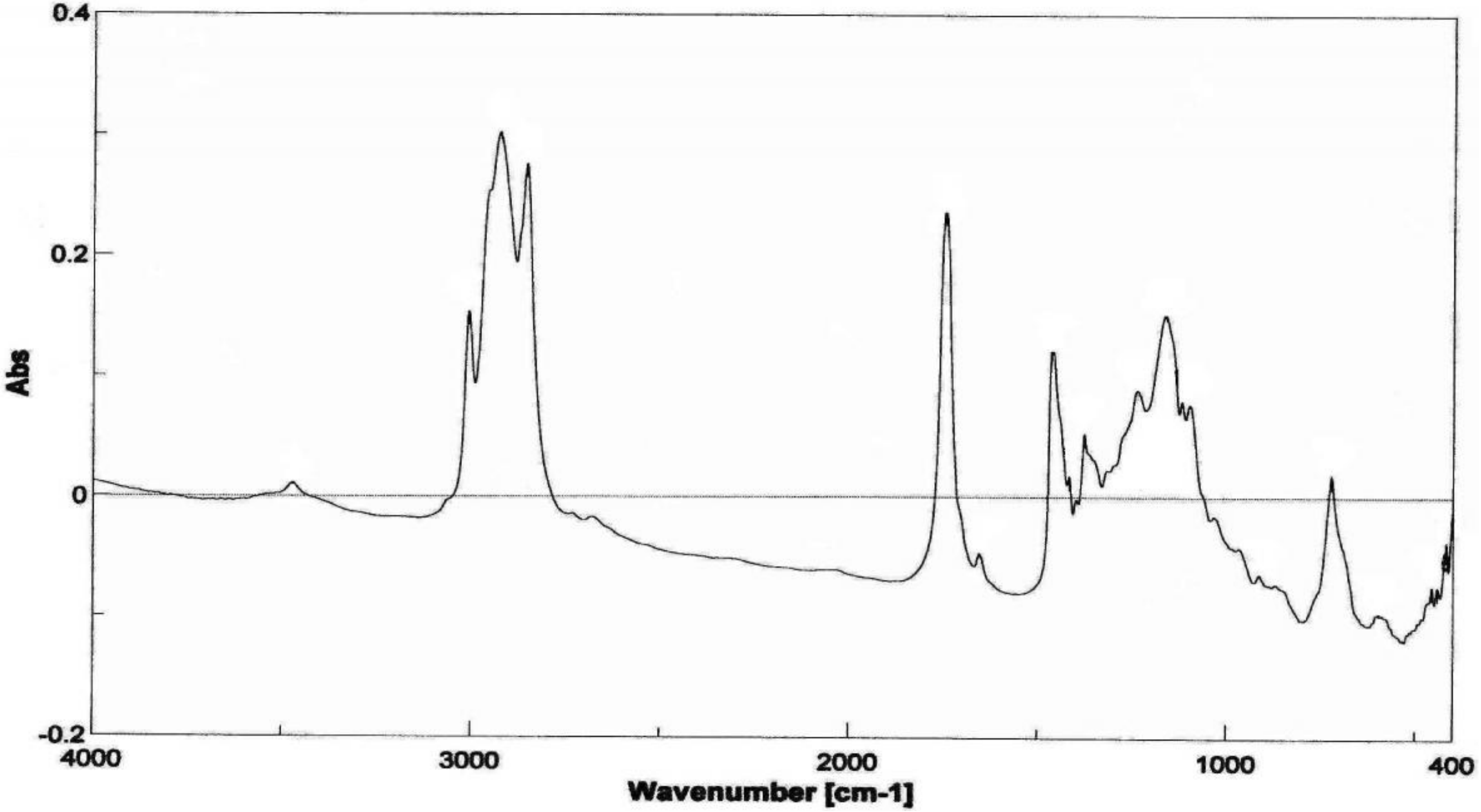

Figure (20) Typical FTIR absorption spectra of 50% corn oil

These observations are due their composition, as vegetable oils contain certain higher proportion of linolenic or linoleic acyl groups whereas extra virgin olive oil contains higher proportion of oleic acyl groups. However, as the corn oil is gradually mixed in various proportions with extra virgin olive oil, the clear shift of the 3005 cm^{-1} band was attributed to the C–H stretching vibration of *cis*- double bond, to the 3008 cm^{-1} band which is noticed.

Also, it was observed a shift of the absorption band at 2926 cm^{-1}, this band is attributed to the symmetric stretching vibration of the aliphatic CH_2 group which primarily showed absorbance at 2925 cm^{-1} in case of extra virgin olive oil represented in Figure (5). This band shifted from 2925 to 2926 cm^{-1} beginning from the concentration of 20% adulterant corn oil of 20%.

Therefore, in order to quantify further the adulteration, the maximum heights of the two bands at 3005 and 2926 cm^{-1} was also used. The height of the 3005 cm^{-1} band for the extra virgin olive oil sample is obviously smaller than it is for all the types of vegetable oils, and changes according to the extent of adulteration. When the percentage of added vegetable oil increases, the height of this band also increases, approaching the height that matches to the vegetable oil. The 2925 cm^{-1} band height exhibits small changes and not in a specific way, as the 3005 cm^{-1} band. The relationship between shift of absorption peak and concentrations of adulterant corn oil was tabulated in Table (20) and plotted in Figure (31) which showed a linear relationship. These observations are in accordance with those reported by **Vlachos et al. (2006).**

Also, a band shift was observed at 914 cm^{-1} concerning the pure corn oil which it was at 912 cm^{-1} in case of extra virgin olive oil. With gradual addition of adulterant corn oil, it was observed that the value of band shifted away from 912 cm^{-1} (characteristic of extra virgin olive oil). It was found an absorbance at 912 cm^{-1} corresponding to 5% corn oil; absorbance at 913 cm^{-1} corresponding to 10% and 20% corn oil; absorbance at 914 cm^{-1} corresponding to 30%, 40% and 50% corn oil.

These findings indicated that the band was shifted away from 912 cm^{-1} (characteristic of extra virgin olive oil) starting from concentration of 10% of the adulterant corn oil which gave absorption at 913 cm^{-1}, but the 5% corn oil was still giving absorption at 912 cm^{-1} which is the same of extra virgin olive oil.

Concerning the absorption peak at 1163 cm^{-1}, it was noticed that there was some increases in the absorption intensity values with gradual addition of adulterant corn oil beginning from the concentration of 5% of adulterant corn oil till pure corn oil. This relationship was tabulated in Table (18) and plotted in Figure (29). The absorption peak at 1163 cm^{-1} is due to C–O stretching and C–H bending. There was no shifts away from this wavenumber in FTIR spectral data, but there exists some variations in absorption intensities with varying the concentration of the adulterant corn oil in such a way to be valuable to be tabulated in Table (18), with its related plot in Figure (29) which shows the relationship between changes in absorption intensity values at 1163 cm^{-1} and the concentrations of adulterant corn oil. It is shown that the absorption intensity of extra virgin olive oil was evaluated as 0.416. With gradual addition of adulterant corn oil concentrations, it was found that the absorption intensity values increased being 0.452, 0.463, 0.503, 0.587, 0.654, 0.743 and 0.959 for the concentrations of 5%, 10%, 20%, 30%, 40%, 50% and 100% corn oil, respectively. This means that it is possible to detect the adulteration of extra virgin olive oil with adulterant corn oil beginning from 5% corn oil using the absorption intensity at 1163 cm^{-1}. These findings are supported by those reported by **Allam and Hamed (2007).**

There exists an important absorption band at 967 cm^{-1} which was found out in FTIR spectra of corn oil and was absent from extra virgin olive oil. This wavenumber along with some absorption intensity was achieved only in pure corn oil. The absorption peak at 967 cm^{-1} was due to C–H out-of-plane deformation band. This band is broad with down sloping baseline because of the overlap of the broad absorption of triglycerides and of the C–H out-of-plane deformation bands arising from the conjugated linoleic acids (CLA) in the sample. Also, the isolated *trans* isomers in oils and fats give rise to a weak absorption around 967 cm^{-1}. The problems encountered in the determination of *trans*- content in fats and oils by infrared spectrometry using the absorption at 967 cm^{-1} lie in the fact that there was no ideal reference material that can remove the sloping of the *trans*- band at 967 cm^{-1}. This is because the triglycerides of fats and oils contain a variety of combinations of fatty acids in the molecules and the absorptions in the infrared spectrum arising from the fatty acid groups are affected by the other fatty acids present in a glyceride molecule. Researchers have been trying to improve the ways to quantify the absorption at 967 cm^{-1} because of the importance of conjugated linoleic acids (CLA) which are a mixture of geometrical and positional isomers of linoleic acid with conjugated double bonds and can be found in partially hydrogenated corn oil which is rich in linoleic acids. The peak at 967 cm^{-1} of FTIR spectra of pure corn oil was due to C=C bending of *trans*- RHC=CHR. These results are in conformity with those reported by **Yang and Irudayaraj (2001); Christy et al. (2003).**

From FTIR spectra of extra virgin olive oil and corn oil, an important peak ascribed to the very weak stretching of the C=O of the acid group located at 1653 cm^{-1} with considerable absorption intensity appears as a shoulder of the very strong band of the C=O stretching band of the ester group located at 1747 cm^{-1}. This peak is considered as an important tool because it represents the acidity of the extra virgin olive oil. When comparing the absorption intensities at 1653 cm^{-1} for extra virgin olive oil alone and with mixtures with corn oil, it was found that it has higher absorption in case of extra virgin olive oil compared with its mixtures with corn oil which interpret the higher acidity of extra virgin olive oil, and this results came in accordance with results obtained from the manual chemical analysis which showed the higher acidity of extra virgin olive oil and the lower acidity obtained from the mixtures of extra virgin olive oil with corn oil at any concentrations of adulterant corn oil. These observations agree with those reported by **Iñón et al. (2003).**

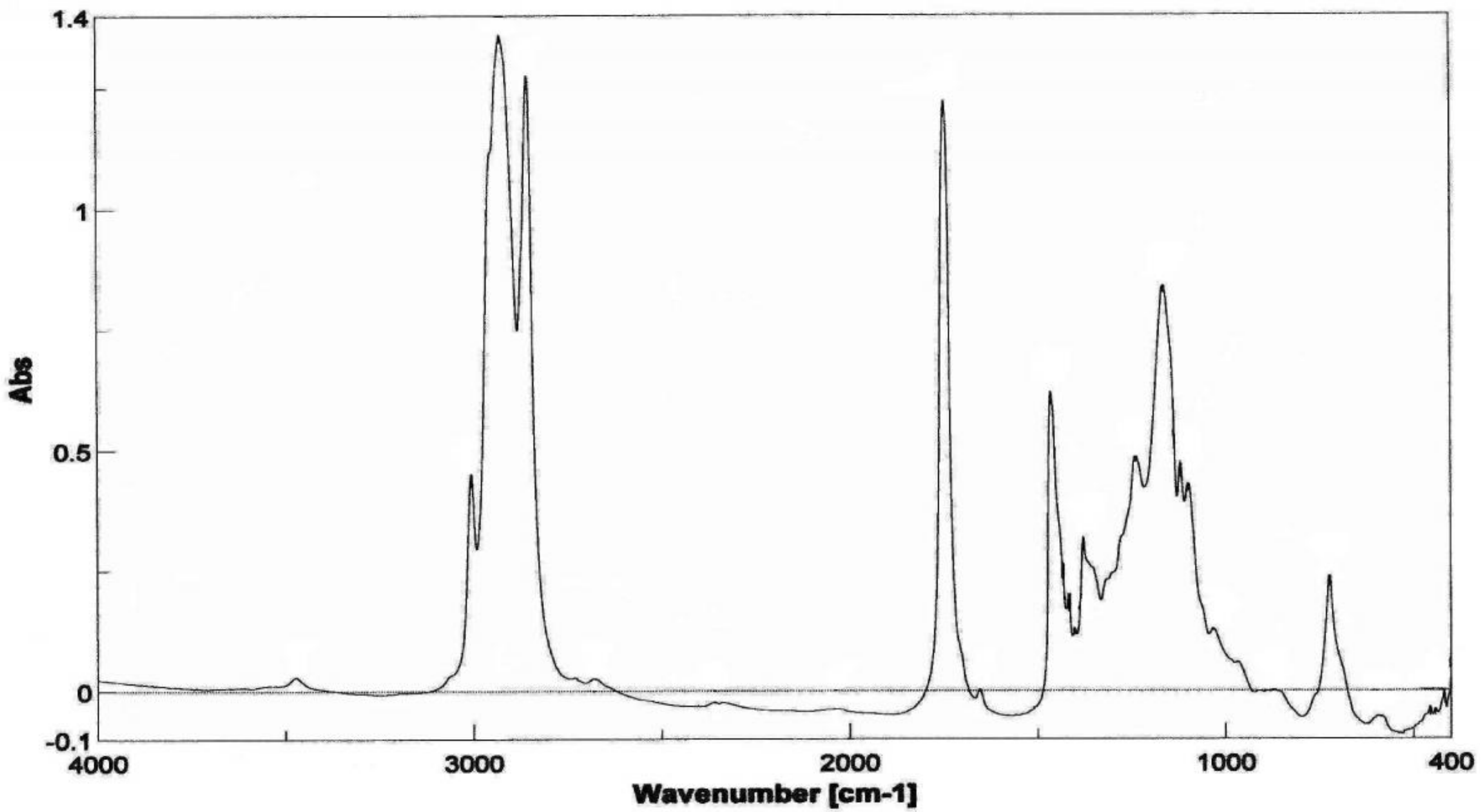

Figure (21) Typical FTIR absorption spectra of 5% refined olive oil

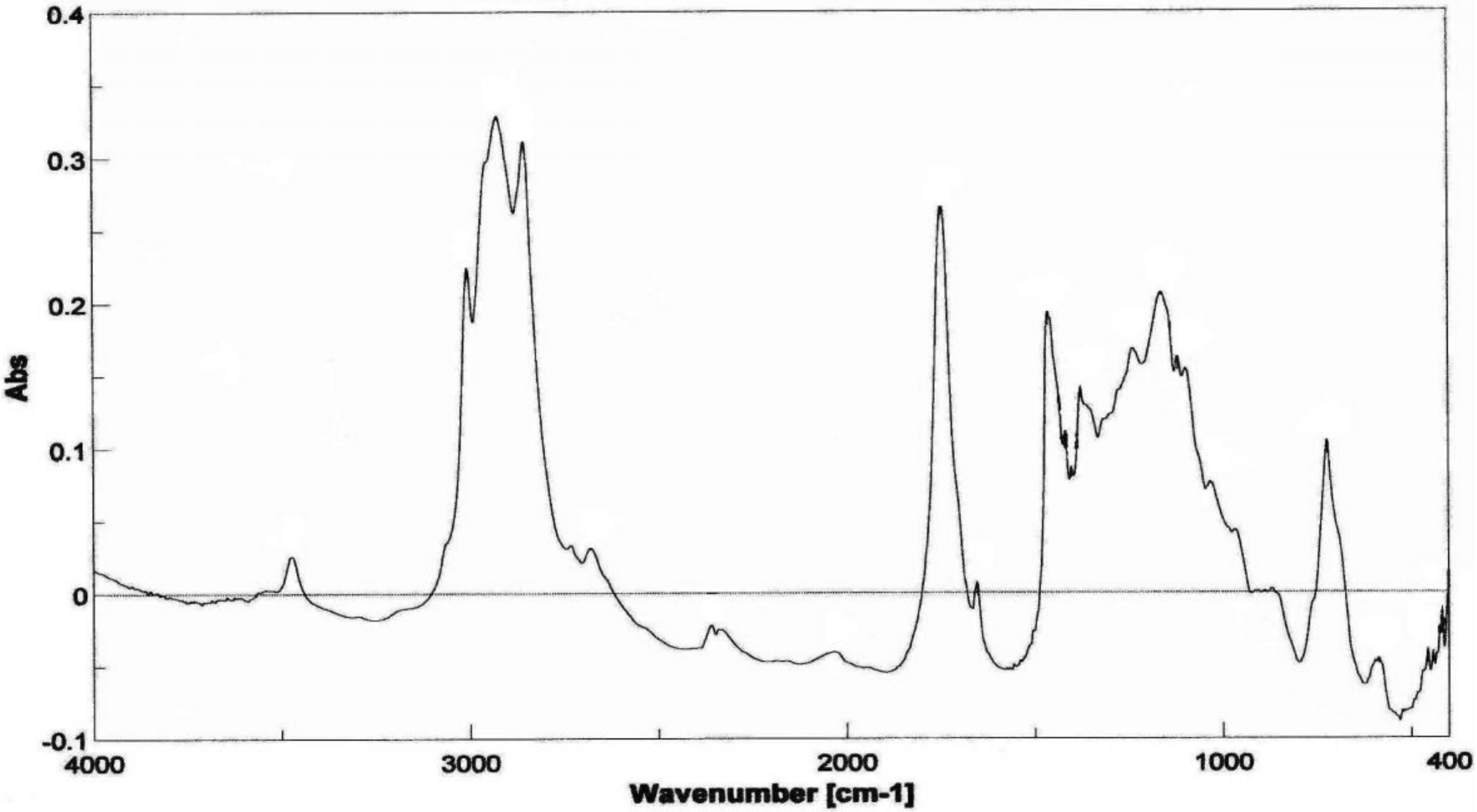

Figure (22) Typical FTIR absorption spectra of 10% refined olive oil

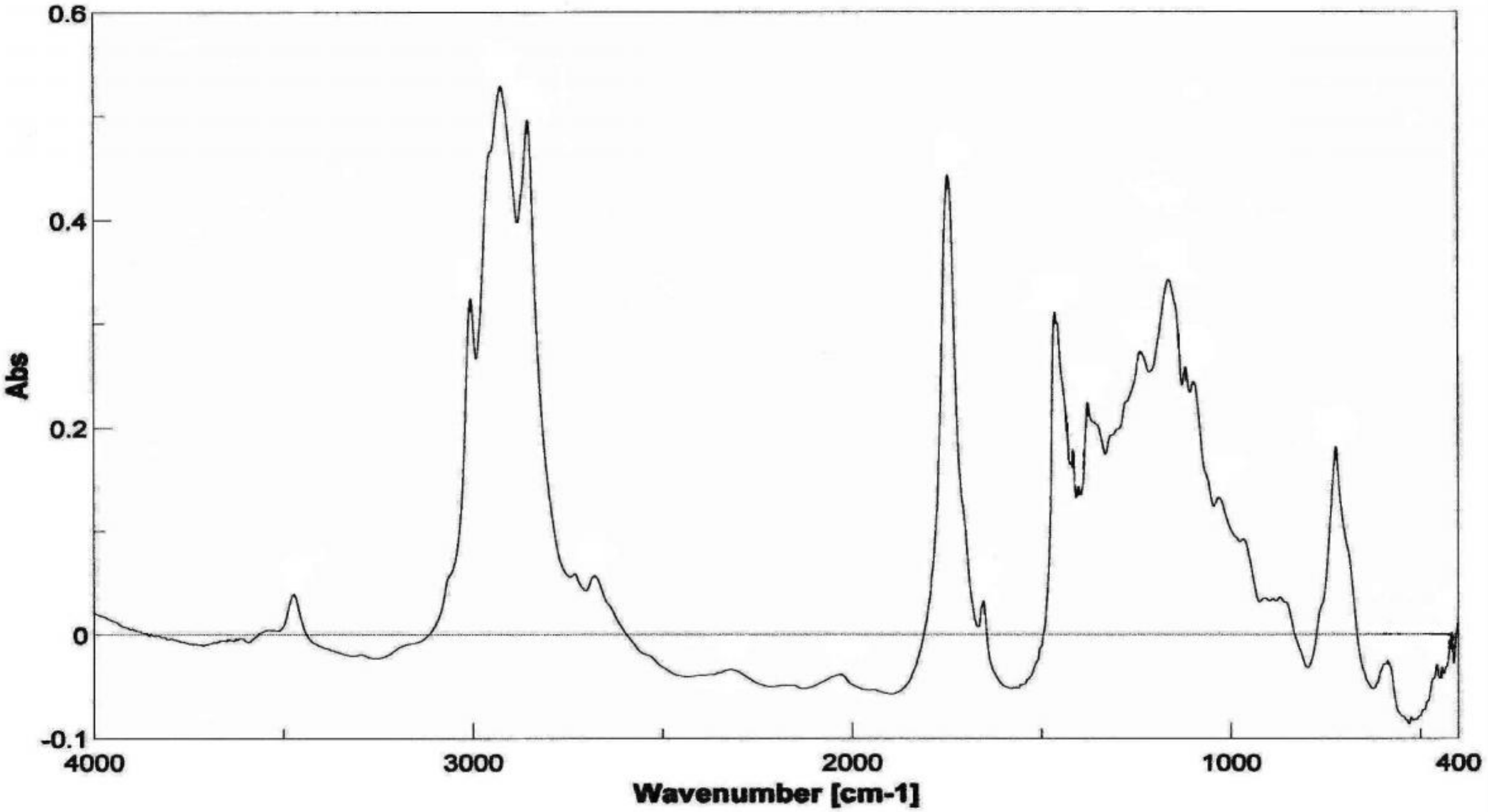

Figure (23) Typical FTIR absorption spectra of 20% refined olive oil

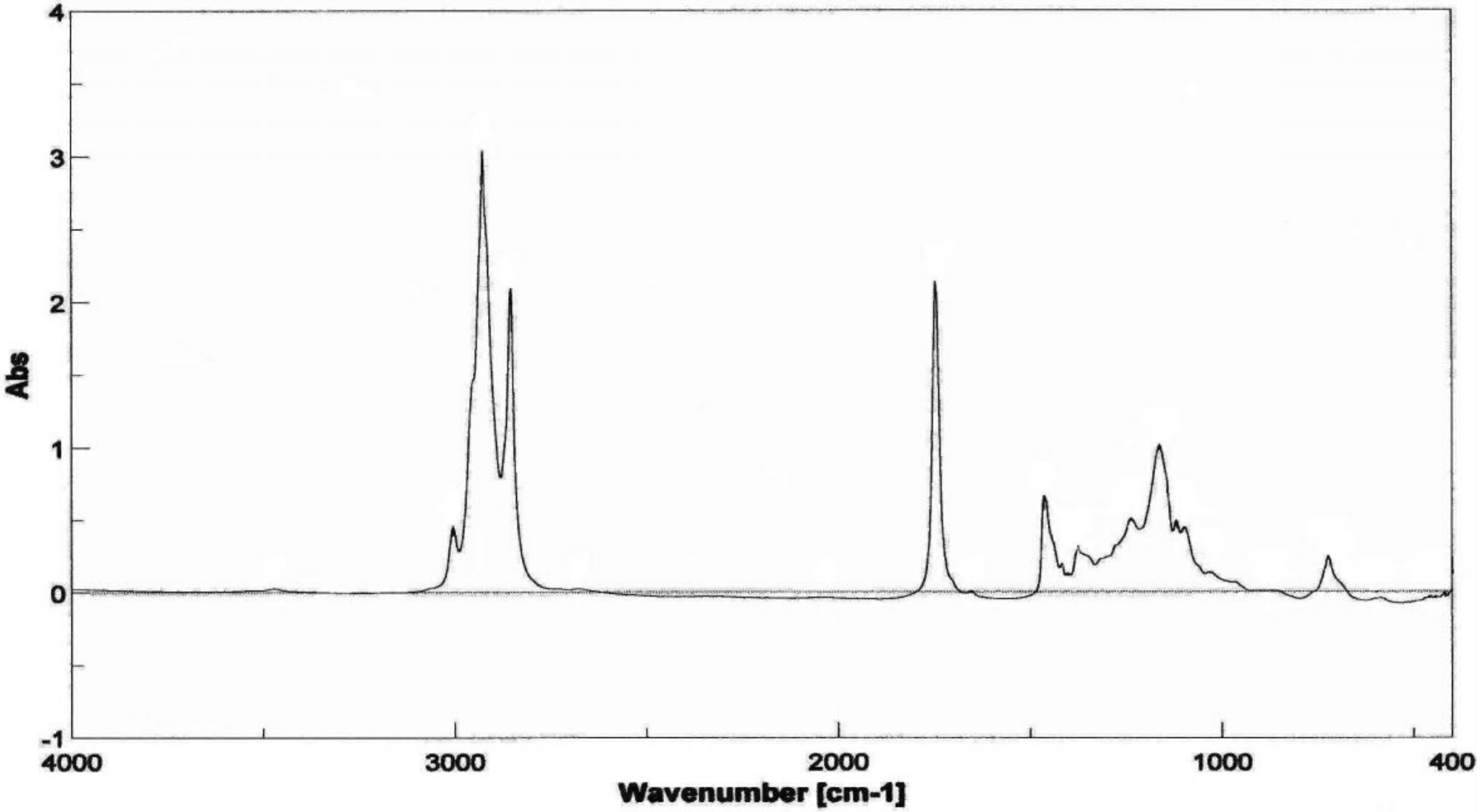

Figure (24) Typical FTIR absorption spectra of 30% refined olive oil

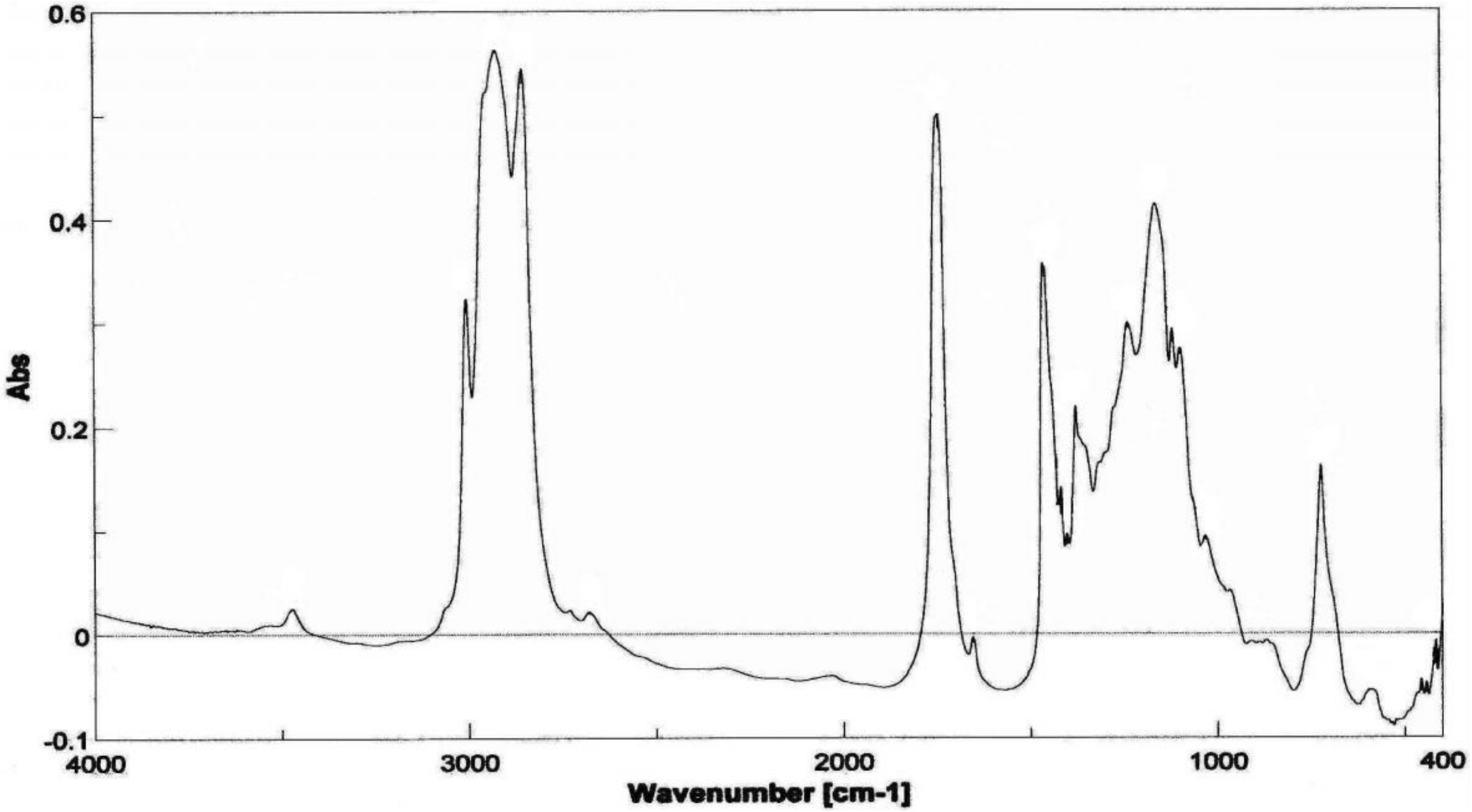

Figure (25) Typical FTIR absorption spectra of 40% refined olive oil

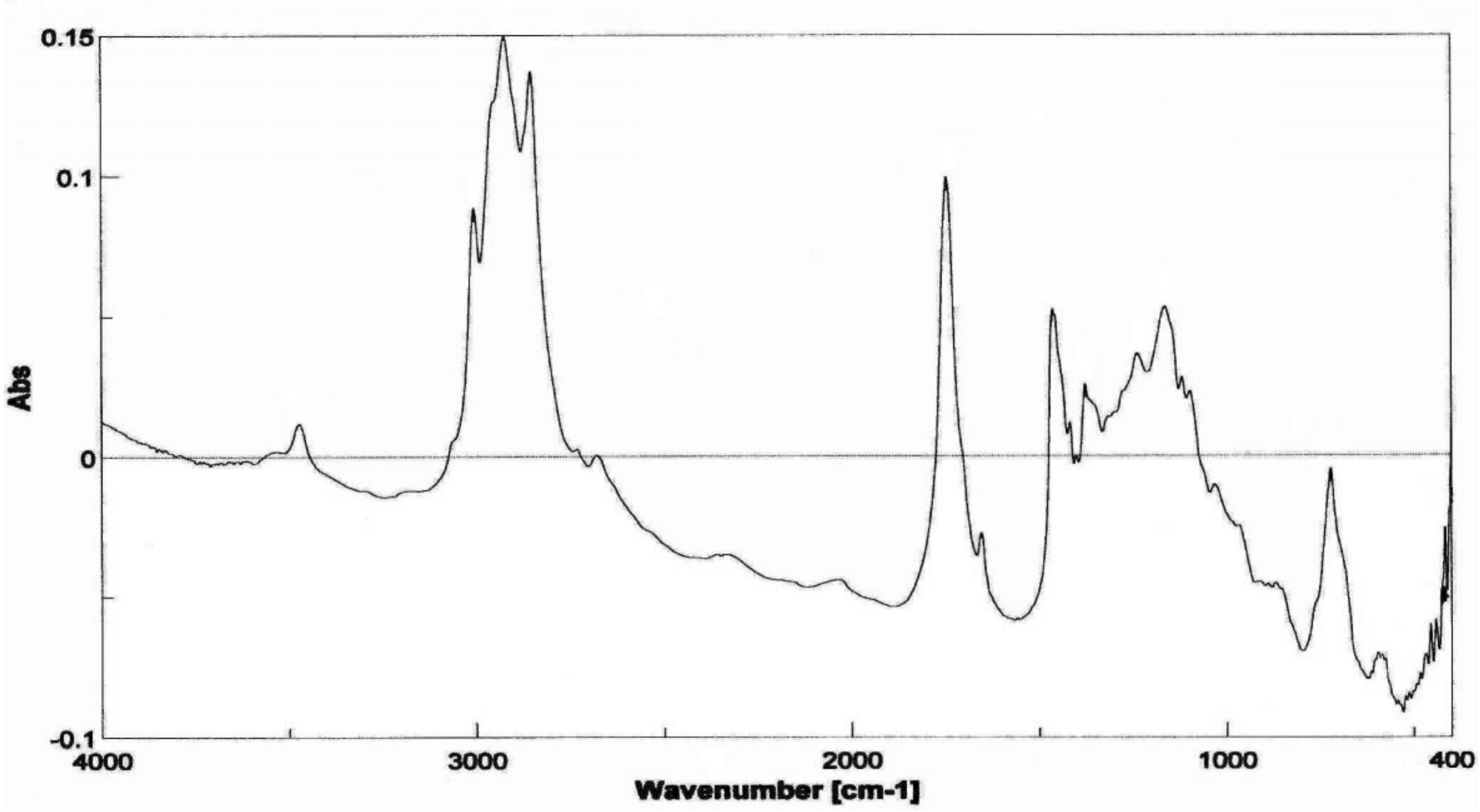

Figure (26) Typical FTIR absorption spectra of 50% refined olive oil

4.4.5- Detection of adulteration of extra virgin olive oil with adulterant refined olive oil using FTIR spectral data analysis:

As shown from Figures (21, 22, 23, 24, 25 and 26), these 6 figures represent FTIR spectral data analysis of the extra virgin olive oil in its binary admixtures with gradual increasing of adulterant refined olive oil at different concentrations of 5, 10, 20, 30, 40 and 50%. As formerly noticed from Figure (8) representing pure refined olive oil, a band shift observed at 2926 cm^{-1}. This band is attributed to the symmetric stretching vibration of the aliphatic CH_2 group which has primarily showed absorbance at 2925 cm^{-1} in case of extra virgin olive oil represented in Figure (5). This band was shifted from 2925 to 2926 cm^{-1} at the beginning of the concentration of adulterant refined olive oil from 30% in the blend. In order to quantify further the adulteration, the maximum heights of the band at 2926 cm^{-1} was used. These observations fell in agreement with those reported by **Vlachos et al. (2006).**

Also, a band shift was observed at 914 cm^{-1} concerning the pure refined olive oil which was 912 cm^{-1} in case of extra virgin olive oil. With gradual addition of adulterant refined olive oil it observed that the value of band was shifted away from 912 cm^{-1} (characteristic of extra virgin olive oil). We discovered absorbance at 912 cm^{-1} corresponding to 5% and 10% refined olive oil; absorbance at 913 cm^{-1} corresponding to 20%, 30% and 40% refined olive oil and absorbance at 914 cm^{-1} corresponding to 50% refined olive oil. This findings indicated that the band was shifted away from 912 cm^{-1} (characteristic of extra virgin olive oil) with starting the concentration of adulterant refined olive oil from 20% which gave absorption at 913 cm^{-1}, but the 5% and 10% refined olive oil were still giving absorption at 912 cm^{-1} which is the same of extra virgin olive oil.

Concerning the absorption peak at 1163 cm^{-1}, it was noticed that there was some increases in the absorption intensity values with gradual addition of adulterant refined olive oil beginning from the concentration of 5% of adulterant refined olive oil till pure refined olive oil. This relationship was tabulated in Table (18) and plotted in Figure (29). There exists an important absorption peak of considerable intensity at 1163 cm^{-1} due to C–O stretching and C–H bending. There was no shifts away from this wavenumber in FTIR spectral data, but there exists some variations in absorption intensities with varying the concentration of the adulterant refined olive oil in such a way to be valuable to be tabulated in Table (18) with its related plot in Figure (29) which shows the relationship between changes in absorption intensity values at 1163 cm^{-1} and the concentrations of adulterant refined olive oil. It was shown that the absorption intensity of extra virgin olive oil was evaluated as 0.416. With gradual addition of adulterant refined olive oil concentrations, it was found that the absorption intensity values were 0.447, 0.492, 0.553, 0.653, 0.741, 0.907 and 1.054 for the concentrations of 5%, 10%, 20%, 30%, 40%, 50% and 100% refined olive oil, respectively. This means that it is possible to detect the adulteration of extra virgin olive oil with adulterant refined olive oil beginning with the concentration of 5% refined olive oil. These obtained results showed an excellent agreement with those reported by **Allam and Hamed (2007).**

A peak ascribed to the very weak stretching of the C=O of the acid group located at 1653 cm^{-1} appears as a shoulder of the very strong band of the C=O stretching band of the ester group located at 1747 cm^{-1}. This peak represents the acidity of the extra virgin olive oil. When comparing the absorption intensities at 1653 cm^{-1} for extra virgin olive oil alone and with its mixtures with refined olive oil, it showed higher absorption in case of extra virgin olive oil compared with its mixtures with refined olive oil which interpret the higher acidity of extra virgin olive oil. This finding agree with the results obtained from the manual chemical analysis used in the determination of the free acidity values of pure extra virgin olive, the binary admixtures with adulterant refined olive oil and the pure refined olive oil; that showed the higher acidity of extra virgin olive oil and the lower acidity obtained from the mixtures of extra virgin olive oil with refined olive oil at any concentrations of adulterant refined olive oil. These results agree with those reported by **Iñón et al. (2003).**

4.4.6- Detection of adulteration of extra virgin olive oil with adulterant oils (sunflower oil, corn oil and refined olive oil) using FTIR spectral data analysis and useful relationships:

There are useful relationships deduced from the observation of FTIR spectral data analysis of extra virgin olive oil and the adulterant oils (sunflower, corn and refined olive oils) added at different concentrations in the binary admixtures. These relationships were selected, plotted graphically and finally have been shown to be of great importance in detecting adulteration of extra virgin olive with adulterant studied oils.

Tables (16, 17, 18, 19 and 20) and Figures (27, 28, 29, 30 and 31) show these useful relationships.

At the wavenumbers; 1118 and 1097 cm^{-1} (assigned to C–O stretching), the values of their absorbance ratio (R1118/1097 cm^{-1}) decreased with increasing the concentrations of added adulterant oils (sunflower, corn and refined olive oils) with an excellent linear relationship beginning from the concentration of 5% of any of the adulterant oils as shown in Table (16) and Figure (27).

At the wavenumbers; 1747 and 2925 cm^{-1} (assigned to C–O stretching), the values of their absorbance ratio (R1747/2925 cm^{-1}) also decreased with increasing the concentrations of added adulterant studied oils with an evident linear relationship beginning from the concentration of 5% of the adulterant oils as shown in Table (17) and Figure (28).

In addition, the absorption intensity values of the spectral bands at 1163 cm^{-1} (assigned to C–O stretching and CH_2– bending) showed apparent increase with increasing the concentrations of added adulterant oils with an excellent linear relationship as shown in Table (18) and Figure (29).

Also, there was a pronounced shift of the peak at 912.78 cm^{-1} (assigned for –HC=CH–, *cis*-, bending out-of-plane) for pure olive oil to higher wave numbers with increasing the concentrations of added adulterant oils with an excellent linear relationship as shown in Table (19) and Figure (30).

There was a pronounced shift of the peak at 3005.52 cm^{-1} assigned to the C–H stretching vibration of the *cis*-double bond (=CH) for pure olive oil to higher wave numbers with increasing the concentrations of added adulterant sunflower and corn oils with an excellent linear relationship. While, this relationship was not evident for the mixtures of extra virgin olive oil with the adulterant refined olive oil, where there was no change was observed and no shift of absorption peak at 3005.52 which may be attributed to the great similarity between extra virgin olive oil and refined olive oil as shown in Table (20) and Figure (31). These results agreed with those reported by **Allam and Hamed (2007).**

It is concluded that the adulteration of extra virgin olive oil can be monitored by using FTIR spectroscopy by measuring the changes in the absorbance ratios; R1747/2925 cm^{-1} and R1118/1097 cm^{-1} to lower values, the changes in the absorption intensity values at 1163.83 cm^{-1} to higher values and the shifts of the absorption peaks at 912.78 and 3005.52 cm^{-1} to higher wavenumbers in the adulterated blends compared with pure extra virgin olive oil.

The detection limit for extra virgin olive oil adulteration with adulterant sunflower, corn and refined olive oils is 5% for the three adulterant oils under investigation which is much lower than the limit at which there exists a threatening of adulteration of extra virgin olive oil. Finally, FTIR spectroscopy proved its potency as a rapid, cheap, nondestructive and authenticity measuring tool.

Table (16) Absorbance ratio values (R1118/1097 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil	Absorbance ratio values (R1118/1097 cm^{-1}) of binary admixtures		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	1.090 (EVOO)	1.090 (EVOO)	1.090 (EVOO)
5	1.054	1.080	1.087
10	1.042	1.066	1.080
20	1.037	1.058	1.079
30	1.034	1.042	1.077
40	1.027	1.033	1.069
50	1.016	1.031	1.065
100	0.939 (SO)	0.992 (CO)	1.030 (ROO)

EVOO; extra virgin olive oil
SO; sunflower oil
CO; corn oil
ROO; refined olive oil

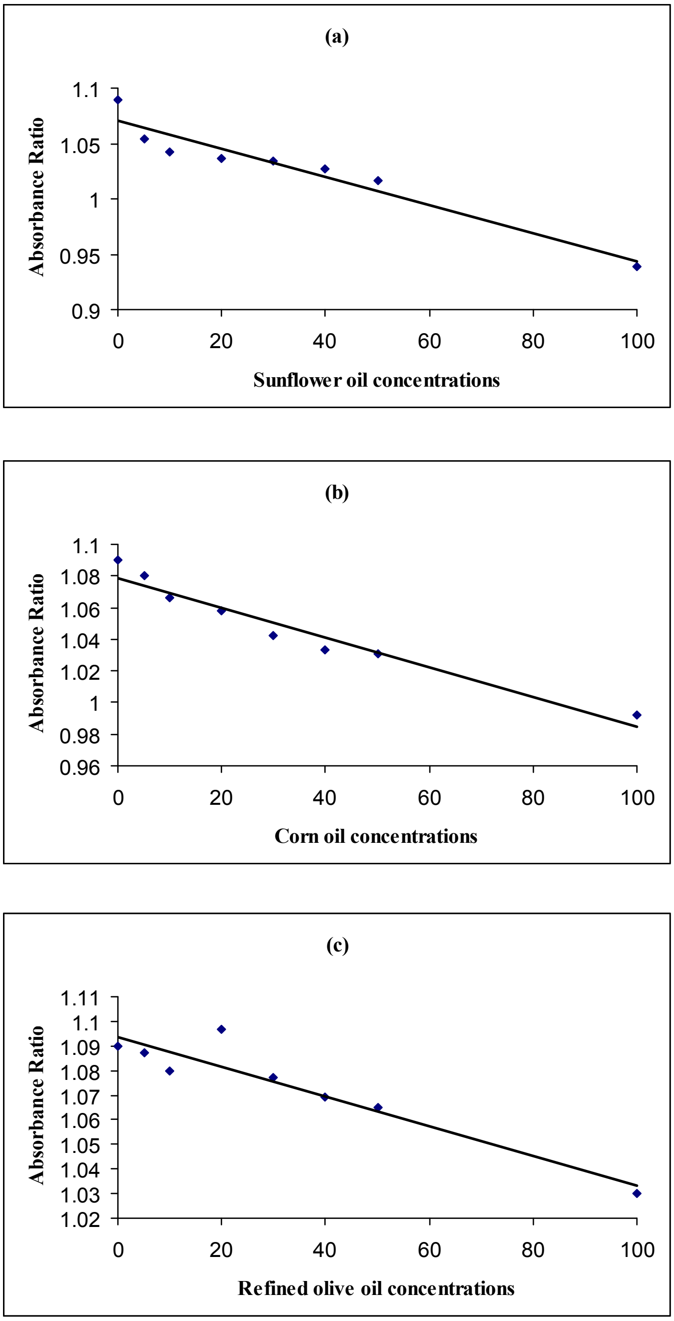

Figure (27) The relationship between changes in Absorbance Ratio (R1118/1097 cm^{-1}) and concentrations of: sunflower oil (a), corn oil (b), and refined olive oil (c)

Table (17) Absorbance ratio values (R1747/2925 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil %	Absorbance ratio values (R1747/2925 cm^{-1}) of binary admixtures		
	EVOO + SO	EVOO + CO	EVOO + ROO
0	0.898 (EVOO)	0.898 (EVOO)	0.898 (EVOO)
5	0.892	0.895	0.887
10	0.882	0.884	0.880
20	0.872	0.873	0.876
30	0.864	0.866	0.870
40	0.853	0.857	0.867
50	0.844	0.845	0.862
100	0.825 (SO)	0.821 (CO)	0.859 (ROO)

EVOO; extra virgin olive oil
SO; sunflower oil
CO; corn oil
ROO; refined olive oil

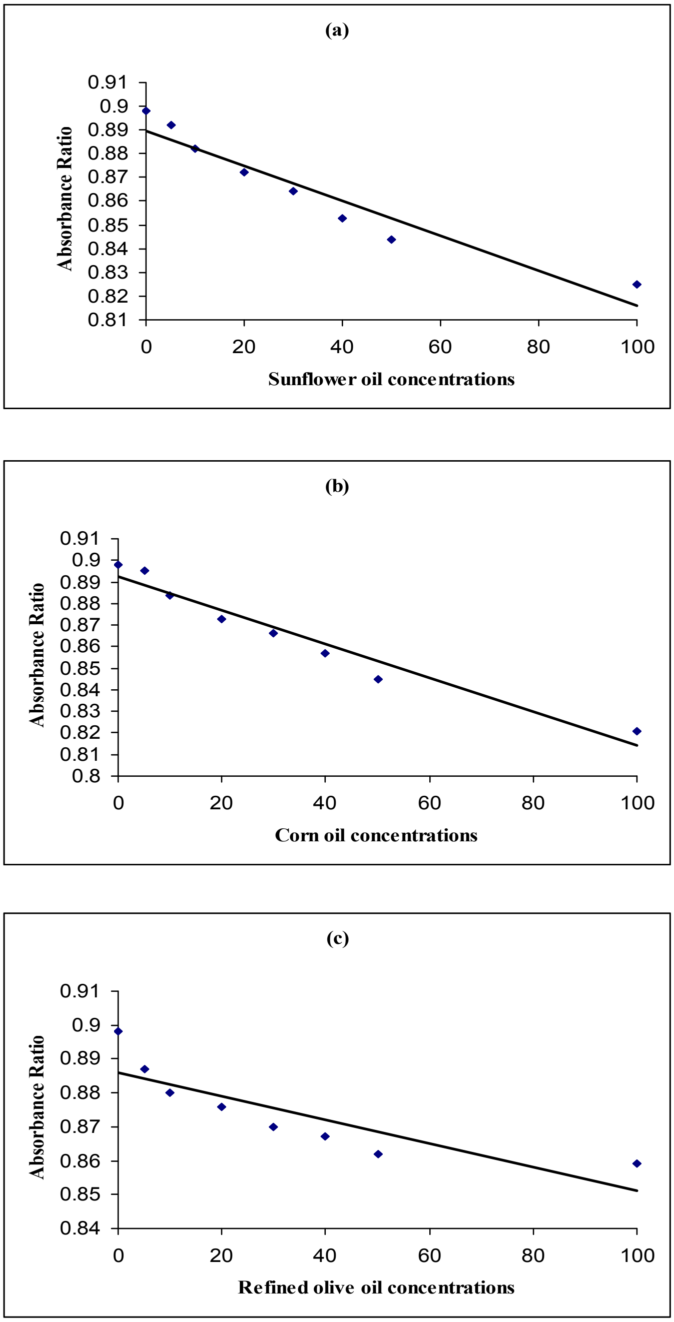

Figure (28) The relationship between changes in Absorbance Ratio ($R1747/2925\ cm^{-1}$) and concentrations of: sunflower oil (a), corn oil (b), and refined olive oil (c)

Table (18) Absorption intensity values (at 1163.83 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil	Absorption intensity values (at 1163.83 cm^{-1}) of binary admixtures		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	0.416 (EVOO)	0.416 (EVOO)	0.416 (EVOO)
5	0.445	0.452	0.447
10	0.455	0.463	0.492
20	0.509	0.503	0.553
30	0.653	0.587	0.653
40	0.708	0.654	0.741
50	0.907	0.743	0.907
100	1.329 (SO)	0.959 (CO)	1.054 (ROO)

EVOO; extra virgin olive oil
SO; sunflower oil
CO; corn oil
ROO; refined olive oil

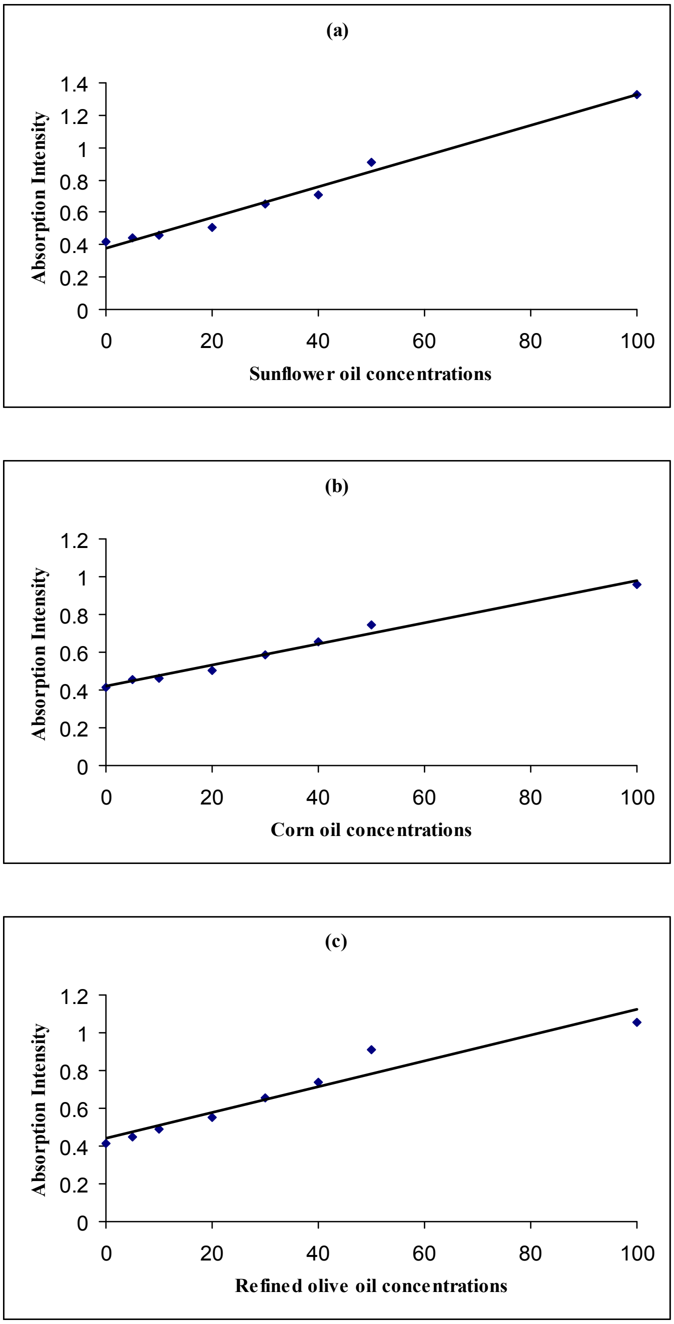

Figure (29) The relationship between changes in Absorption Intensity values (at 1163.83 cm^{-1}) and concentrations of: sunflower oil (a), corn oil (b), and refined olive oil (c)

Table (19) Shift of absorption peak (at 912.78 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil	Shift of absorption peak (at 912.78 cm^{-1}) of binary admixtures		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	912.780 (EVOO)	912.780 (EVOO)	912.780 (EVOO)
5	912.780	912.780	912.780
10	913.129	913.129	912.780
20	913.129	913.129	913.129
30	913.129	914.093	913.129
40	913.129	914.093	913.129
50	914.093	914.093	914.093
100	914.093 (SO)	914.093 (CO)	914.093 (ROO)

EVOO; extra virgin olive oil
SO; sunflower oil
CO; corn oil
ROO; refined olive oil

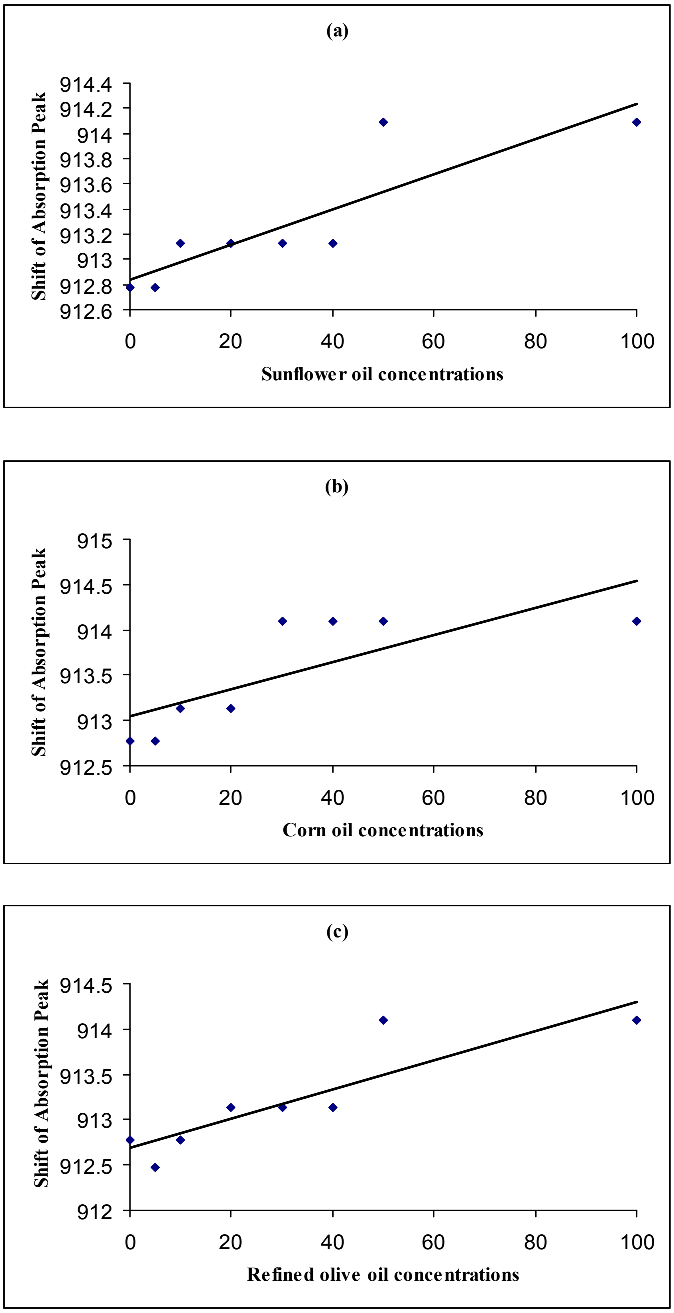

Figure (30) The relationship between Shift of Absorption Peak (at 912.78 cm^{-1}) and concentrations of: sunflower oil (a), corn oil (b), and refined olive oil (c)

Table (20) Shift of absorption peak (at 3005.52 cm^{-1}) of extra virgin olive oil in binary admixtures with different adulterant oils (sunflower oil, corn oil and refined olive oil) at various concentrations

Adulterant oil	Shift of absorption peak (at 3005.52 cm^{-1}) of binary admixtures		
%	EVOO + SO	EVOO + CO	EVOO + ROO
0	3005.52 (EVOO)	3005.52 (EVOO)	3005.52 (EVOO)
5	3005.52	3005.52	3005.52
10	3005.52	3005.52	3005.52
20	3006.48	3006.48	3005.52
30	3006.48	3006.48	3005.52
40	3007.44	3007.44	3005.52
50	3007.44	3007.44	3005.52
100	3008.41 (SO)	3008.41 (CO)	3005.52 (ROO)

EVOO; extra virgin olive oil

SO; sunflower oil

CO; corn oil

ROO; refined olive oil

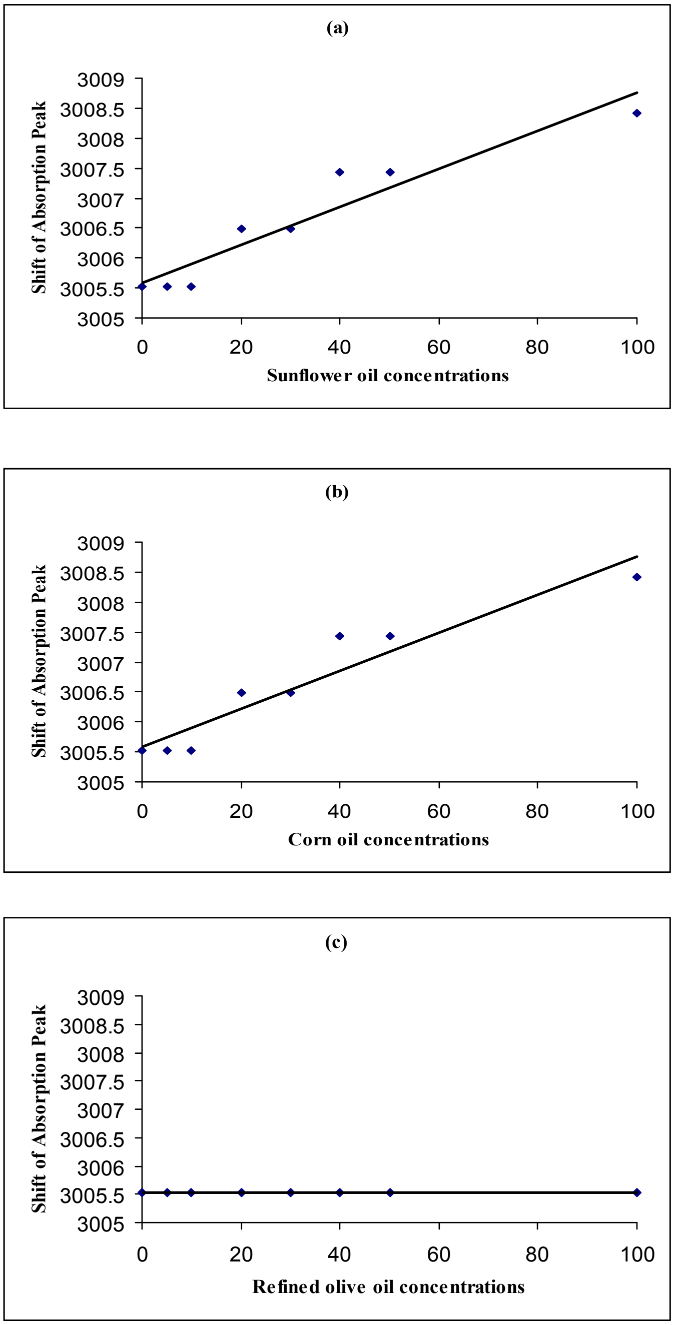

Figure (31) The relationship between Shift of Absorption Peak (at 3005.52 cm^{-1}) and concentrations of: sunflower oil (a), corn oil (b), and refined olive oil (c)

5- SUMMARY

Determination of authenticity of extra virgin olive oils has become very important in recent years due to the increasing public concerns about possible adulterations with relatively cheap vegetable oils such as sunflower oil, soybean oil, sesame oil, corn oil and refined olive oil.

This work aims at monitoring of extra virgin olive oil quality in order to ensure the authenticity of this vital consumer product and to reveal any adulteration may occur in this product by applying a cheap nondestructive recent method (FTIR spectroscopy) which is time saving and does not need to proceed through sophisticated sample preparations.

Extra virgin olive oil was extracted from fresh olive fruits by Oliomio-machine then filtered and kept in brown glass bottles at -5^{o}C. Refined olive oil, sunflower oil and corn oil samples were purchased locally. According to their labels, all the oils used in adulteration were additive-free, refined, bleached and deodorized (RBD).

Different binary admixtures of extra virgin olive oil with each of the adulterant oils were prepared for the purpose of investigations.

Sunflower, corn, and refined olive oils (as adulterant oils) were used to make blends with extra virgin olive oil at different concentrations of 0, 5, 10, 20, 30, 40, 50, and 100% (w/w).

Both physical and chemical evaluations were done as follows: refractive index, free fatty acid, peroxide value, iodine value, UV-absorption characteristics (conjugated diene, conjugated triene and ΔK).

- The obtained data were compared with the standard values reported by IOOC Standards and Codex Alimentarius Standard.
- Fatty acids and unsaponifiable matters were also investigated by capillary gas chromatography separation techniques and identified by matching with standards.
- Besides, Fourier transform infrared spectroscopy was applied by simply measuring the absorbance spectra of the admixtures.

The obtained results are summarized as follows:

- Refractive Index (RI) is considered as a valuable tool for detecting adulteration. Addition of different concentrations from adulterant oils (5, 10, 20, 30, 40, and 50%) to extra virgin olive oil showed gradual increases in refractive index (RI) of these blends. This observation was noticed in the case of addition of the same concentrations either from sunflower, corn or refined olive oils to extra virgin olive oil since they have a higher refractive index; being 1.4722, 1.4755 and 1.4705 for sunflower, corn and refined olive oils, respectively, than that of extra virgin olive oil (1.4678).
- An admixture contained 30% sunflower oil caused the blend out of range of Codex Alimentarius Standard which showed that the refractive index (RI) of extra virgin olive oil and refined olive oil ranged from 1.4677 to 1.4705. While a mixture containing 20% corn oil caused the same finding. Whereas, the adulteration with refined olive oil could not be detected at any concentrations using the refractive index parameter.
- Concerning the acid values (AV) of extra virgin olive oil blended with the adulterant oils under investigations, it was clear that although the addition of different concentrations from each of sunflower, corn and refined olive oils (5, 10, 20, 30, 40, and 50%) to extra virgin olive oil showed gradual decreases in the acid values of these blends starting from pure extra virgin olive oil till pure sunflower, corn and refined olive oils, with these decrements the blends were still present inside the range of acid value of extra virgin olive oil reported by Codex Alimentarius Standard and IOOC. This means that the adulteration of extra virgin olive oil with the adulterant oils under investigations can not be detected at any concentration by measuring acid values.
- Similarly, peroxide values (PV) of extra virgin olive oil in binary admixtures with adulterant oils under investigations can not detect the adulteration of extra virgin olive oil with the adulterant oils at

any concentrations because the values still present in the range established by Codex Alimentarius Standard and IOOC.

- Adulteration of extra virgin olive oil by determination of iodine value could be detected in blends containing 30% upward higher concentrations of adulterant sunflower oil or corn oil. Whereas, the adulteration with refined olive oil could not be detected at any concentrations using the iodine value parameter.
- Results revealed that the adulteration will be detected easily by UV absorbance at 270 nm because sunflower oil showed a shift out of the permitted range at 20% upward higher concentrations added to extra virgin olive oil. Also, corn oil will be detected if present in 5% upward higher concentrations in blends with extra virgin olive oil. However, refined olive oil will be detected if present in 10% upward higher concentrations with extra virgin olive oil.
- The calculated ΔK allowed the detection of adulteration in extra virgin olive oil at the concentration of 5% upward higher concentrations of adulterant sunflower, corn and refined olive oils.
- Results revealed that using of oleic, linoleic, and linolenic acids percentages obtained by GC analysis could be used as a powerful tool to detect the adulteration of extra virgin olive oil with sunflower, corn and refined olive oils at concentrations of 30%, 30% and 20% for the mentioned three adulterant oils, respectively.
- The spectral region (1300-1000 cm^{-1}) which contains the IR fingerprints of these vegetable oils was found to be very useful in detecting olive oil adulteration.
- A band shift observed around 3009 cm^{-1} assigned to the =C–H stretching vibration of the *cis*-double bond, allows the determination of extra virgin olive oil adulteration. This band at this wavenumber was not showed by extra virgin olive oil which has absorption band at 3005.52 cm^{-1}.

- The intensities of the spectral bands at 1163.83 cm^{-1} (assigned to C–O stretching vibration and C–H bending vibration) increased with increasing adulterant oil concentration for each of the three used oils. Whereas, the absorbance ratios (R1747/2925 and R1118/1097 cm^{-1}) decreased with increasing adulterant concentration.
- From the comparison of spectral data of pure oils, there were notable differences in the band around 3005.52 cm^{-1} assigned to C–H stretching vibration of *cis*- double bond (=C–H). The oil composition affects the exact position of the band and yields shifts when the proportion of fatty acid changes. Sunflower and corn oils showed exactly identical absorbance at 3008.41 cm^{-1}, compared to extra virgin olive and refined olive oils which had exactly identical absorbance at 3005.52 cm^{-1}.
- In addition to the above mentioned changes, there was another important measure noticed from the spectral data, this is the shift in position of the peak at 912.78 cm^{-1} (assigned to HC=CH, *cis*-, bending out-of-plane) for extra virgin olive oil to higher wave numbers: 914.093, 914.093, and 914.093 cm^{-1} with the increase of concentration of the adulterant sunflower, corn, and refined olive oils, respectively.
- It is concluded that FTIR spectroscopy has been shown to be the most powerful tool applied easily to detect adulteration in extra virgin olive oil in very low concentrations of the adulterant oil of 5%. This is much lower than the adulteration level at which such adulteration becomes economically significant. Finally, FTIR spectroscopy proved its potency as a rapid, cheap, nondestructive and authenticity measuring tool.

6- REFERENCES

Abd El-Fattah, M.A. (2007). Chemical and biological studies on canola oil. M.Sc. Thesis, Fac. of Agric., Cairo Univ.

Abd El-Razek, A.G. (1998). Studies on chemical composition of some vegetable oil admixtures. M.Sc. Thesis, Fac. of Agric., Cairo Univ.

Abidi, S.L. (2001). Chromatographic analysis of plant sterols in foods and vegetable oils. Journal of Chromatography A, 935: 173–201**.**

Aguilera, M.P., G. Beltrán, D. Ortega, A. Fernández, A. Jiménez and M. Uceda (2005). Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. Food Chemistry, 89: 387-391.

Ahmed, F.H.A. (2004). Biochemical studies on some vegetable oils. Ph.D. Thesis, Fac. of Agric., Cairo Univ.

Allalout, A., D. Krichène, K. Methenni, A. Taamalli, I. Oueslati, D. Daoud and M. Zarrouk (2009). Characterization of virgin olive oil from Super Intensive Spanish and Greek varieties grown in northern Tunisia. Scientia Horticulturae, 120: 77–83.

Allam, M.A. and S.F. Hamed (2007). Application of FTIR spectroscopy in the assessment of olive oil adulteration. Journal of Applied Sciences Research, 3: 102-108.

Alves, M.R., S.C. Cunha, J.S. Amaral, J.A. Pereira and M.B. Oliveira (2005). Classification of PDO olive oils on the basis of their sterol composition by multivariate analysis. Analytica Chimica Acta, 549: 166-178.

AOAC (2000). Association of Official Analytical Chemists. Official methods of analysis. 17th Edition, Volume II, Published by AOAC International, USA.

Aranda, F., S. Gómez-Alonso, R.M. Rivera del Álamo, M.D. Salvador and G. Fregapane (2004). Triglyceride, total and 2-position fatty acid composition of Cornicabra virgin olive oil: comparison with other Spanish cultivars. Food Chemistry, 86: 485-492.

Armenta, S., S. Garrigues, and M. de la Guardia (2007). Determination of edible oil parameters by near infrared spectrometry. Analytica Chimica Acta, 596: 330-337.

Awatif, I.I., M.F. Khalil and H.A.A. Badawy (1996). Effect of natural and synthetic antioxidants on the stability of virgin olive oil, Annals of Agricultural Science, Ain Shams Univ., 41: 269-280.

Baccouri, O., M. Guerfel, B. Baccouri, L. Cerretani, A. Bendini, G. Lercker, M. Zarrouk and D. D. Ben Miled (2008). Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. Food Chemistry, 109: 743-754.

Baeten, V., M. Meurens, M.T. Morales and R. Aparicio (1996). Detection of virgin olive oil adulteration by Fourier transform Raman spectroscopy. Journal of Agricultural and Food Chemistry, 44: 2225-2230.

Barthus, R.C. and R.J. Poppi (2001). Determination of the total unsaturation in vegetable oils by Fourier transform Raman spectroscopy and multivariate calibration. Vibrational Spectroscopy, 26:99-105.

Ben Temime, S., E. Campeol, P.L. Cioni, D. Daoud and M. Zarrouk (2006). Volatile compounds from Chétoui olive oil and variations induced by growing area. Food Chemistry, 99: 312-325.

Ben Temime, S., H. Manai, K. Methenni, B. Baccouri, L. Abaza, D. Daoud, J. S. Casas, E. O. Bueno and M. Zarrouk (2008). Sterolic composition of Chétoui virgin olive oil: Influence of geographical origin. Food Chemistry, 110: 368-374.

Bertran, E., M. Blanco, J. Coello, H. Iturriaga, S. Maspoch and I. Montoliu (1999). Determination of olive oil free fatty acid by Fourier Transform Infrared Spectroscopy. Journal of the American Oil Chemists Society, 76: 611-616.

Bešter, E., B. Butinar, M. Bučar-Miklavčič and T. Golob (2008). Chemical changes in extra virgin olive oils from Slovenian Istra after thermal treatment. Food Chemistry, 108: 446-454.

Bilancia, M.T., F. Caponio, E. Sikorska, A. Pasqualone, and C. Summo (2007). Correlation of triacylglycerol oligopolymers and oxidised triacylglycerols to quality parameters in extra virgin olive oil during storage. Food Research International, 40: 855-861.

Blasi, F., L. Cossignani, M.S. Simonetti and P. Damiani (2007). Biocatalyzed synthesis of *sn*-1,3-diacylglycerol oil from extra virgin olive oil. Enzyme and Microbial Technology, 41: 727-732.

Bonastre, A., R. Ors and M. Peris (2004). Advanced automation of a flow injection analysis system for quality control of olive oil through the use of a distributed expert system. Analytica Chimica Acta, 506: 189-195.

Bortoleto, G.G., L.C.M. Pataca and M.I.M.S. Bueno (2005). A new application of X-ray scattering using principal component analysis – classification of vegetable oils. Analytica Chimica Acta, 539: 283–287.

Boselli, E., G.D. Lecce, R. Strabbioli, G. Pieralisi and N.G. Frega (2009). Are virgin olive oils obtained below 27°C better than those produced at higher temperatures? LWT-Food Science and Technology, 42: 748-757.

Bouaziz, M., I. Fki, H. Jemai, M. Ayadi and S. Sayadi (2008). Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chemistry, 108: 253-262.

Brodnjak-Vončina, D., Z.C. Kodba and M. Novič (2005). Multivariate data analysis in classification of vegetable oils characterized by the content of fatty acids. Chemometrics and Intelligent Laboratory Systems, 75: 31–43.

Cañabate-Díaz, B., A.S. Carretero, A. Fernández-Gutiérrez, A.B. Vega, A.G. Frenich, J.L.M. Vidal and J.D. Martos (2007). Separation and determination of sterols in olive oil by HPLC-MS. Food Chemistry, 102: 593–598.

Casas, J.S., E.O. Bueno, A.M.M. García and M.M. Cano (2004). Sterol and erythrodiol + uvaol content of virgin olive oils from cultivars of Extremadura (Spain). Food Chemistry, 87: 225-230.

Cercaci, L., M.T. Rodriguez-Estrada and G. Lercker (2003). Solid-phase extraction-thin-layer chromatography-gas chromatography method for the detection of hazelnut oil in olive oils by determination of esterified sterols. Journal of Chromatography A, 985: 211-220.

Chiavaro, E., M.T. Rodriguez-Estrada, C. Barnaba, E. Vittadini, L. Cerretani and A. Bendini (2008a). Differential scanning calorimetry: A potential tool for discrimination of olive oil commercial categories. Analytica Chimica Acta, 625: 215-226.

Chiavaro, E., E. Vittadini, M.T. Rodriguez-Estrada, L. Cerretani and A. Bendini (2008b). Differential scanning calorimeter application to the detection of refined hazelnut oil in extra virgin olive oil. Food Chemistry, 110: 248–256.

Chiavaro, E., C. Barnaba, E. Vittadini, M. T. Rodriguez-Estrada, L. Cerretani and A. Bendini (2009). Microwave heating of different commercial categories of olive oil: Part II. Effect on thermal properties. Food Chemistry, 115: 1393-1400.

Christopoulou, E., M. Lazaraki, M. Komaitis and K. Kaselimis (2004). Effectiveness of determination of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. Food Chemistry, 84: 463-474.

Christy, A.A. and P.K. Egeberg (2006). Quantitative determination of saturated and unsaturated fatty acids in edible oils by infrared spectroscopy and chemometrics. Chemometrics and Intelligent Laboratory Systems, 82: 130 – 136.

Christy, A.A., P.K. Egeberg and E.T. Østensen (2003). Simultaneous quantitative determination of isolated *trans* fatty acids and conjugated linoleic acids in oils and fats by chemometric analysis of the infrared profiles. Vibrational Spectroscopy, 33: 37–48.

Christy, A.A., S. Kasemsumran, Y. Du and Y. Ozaki (2004). The detection and quantification of adulteration in olive oil by near-infrared spectroscopy and chemometrics. Analytical Sciences, 20: 935-940.

Clodoveo, M.L., D. Delcuratolo, T. Gomes and G. Colelli (2007). Effect of different temperatures and storage atmospheres on *Coratina* olive oil quality. Food Chemistry, 102: 571-576.

Codex Alimentarius Standard (1999). Codex standard for named vegetable oils. CODEX STAN 210-1999. Available online at http://www.codexalimentarius.net.

Codex Alimentarius Standard (2003). Codex standard for olive oils and olive -pomace oils. CODEX STAN 33-1981 (Rev. 2-2003). Available online at http://www.codexalimentarius.net.

Corn Refiners Association (2006). Corn Oil**.** 5th edition, 1701 Pennsylvania Avenue, N.W. Washington, USA.

Cosio, M.S., D. Ballabio, S. Benedetti and C. Gigliotti (2006). Geographical origin and authentication of extra virgin olive oils by an electronic nose in combination with artificial neural networks. Analytica Chimica Acta, 567: 202-210.

Cunha, S.C. and M.B.P.P. Oliveira (2006). Discrimination of vegetable oils by triacylglycerols evaluation of profile using HPLC/ELSD. Food Chemistry, 95: 518–524.

De Greyta, W., A. Kinta, M. Kellensb and A. Huyghebaerta (1998). Determination of low *trans* levels in refined oils by Fourier transform infrared spectroscopy. Journal of the American Oil Chemists Society, 75: 115-118.

D'Imperio, M., G. Dugo, M. Alfa, L. Mannina and A.L. Segre (2007). Statistical analysis on Sicilian olive oils. Food Chemistry, 102: 956–965.

Dizge, N., C. Aydiner, D.Y. Imer, M. Bayramoglu, A. Tanriseven and B. Keskinler (2009). Biodiesel production from sunflower, soybean, and waste cooking oils by transesterification using lipase immobilized onto a novel microporous polymer. Bioresource Technology, 100: 1983–1991.

Dourtoglou, V.G., T. Dourtoglou, A. Antonopoulos, E. Stefanou, S. Lalas and C. Poulos (2003). Detection of olive oil adulteration using principal component analysis applied on total and regio FA content. Journal of the American Oil Chemists Society, 80: 203-208.

Egyptian Standards (2005a). ES: 49-2/2005, vegetable edible oils, part: 2; olive oils and olive-pomace oils. Egyptian Organization for Standardization and Quality, Egypt.

Egyptian Standards (2005b). ES: 49-7/2005, vegetable edible oils, part: 7; sunflower oil. Egyptian Organization for Standardization and Quality, Egypt.

El-Nikeety, M.M.A. (1981). Chemical and physical changes during oxidation and interesterification of vegetable oils. Ph.D. Thesis, Fac. of Agric., Cairo Univ.

El-Qadi, H. (1976). Chemical studies on olive oil, its cake and analogous oil. M.S. Thesis, Biochemistry Dep., Fac. of Agric., Cairo Univ.

El-Sharkawy, A.A., R.M. El-Mahdy, A.I. Ismael and A.S. Dabash (1984). Studies on the oils of three olive varieties. Annals of Agricultural Science, Fac. of Agric., Ain Shams Univ., 29: 831-840.

Espínola, F., M. Moya, D.G. Fernández and E. Castro (2009). Improved extraction of virgin olive oil using calcium carbonate as coadjuvant extractant. Journal of Food Engineering, 92: 112-118.

Esti, M., M. Contini, E. Moneta and F. Sinesio (2009). Phenolics compounds and temporal perception of bitterness and pungency in extra virgin olive oils: changes occurring throughout storage. Food Chemistry, 113: 1095–1100.

Favier, J.P., D. Bicanic, J. Cozijnsen, B.V. Veldhuizen and P. Helander (1998). CO_2 laser infrared optothermal spectroscopy for quantitative adulteration studies in binary mixtures of extra-virgin olive oil. Journal of the American Oil Chemists Society, 75: 359-362.

Flores, G., M.L. R. del Castillo, M. Herraiz and G.P. Blanch (2006a). Study of the adulteration of olive oil with hazelnut oil by online coupled high performance liquid chromatographic and gas chromatographic analysis of filbertone. Food Chemistry, 97: 742–749.

Flores, G., M.L. R. del Castillo, G.P. Blanch and M. Herraiz (2006b). Detection of the adulteration of olive oils by solid phase microextraction and multidimensional gas chromatography. Food Chemistry, 97: 336–342.

Galtier, O., N. Dupuy, Y. Le Dréau, D. Ollivier, C. Pinatel, J. Kister and J. Artaud (2007). Geographic origins and compositions of virgin olive oils determinated by chemometric analysis of NIR spectra. Analytica Chimica Acta, 595: 136-144.

Gamazo-Vázquez, J., M.S. García-Falcón and J. Simal-Gándara (2003). Control of contamination of olive oil by sunflower seed oil in bottling plants by GC-MS of fatty acid methyl esters. Food Control, 14: 463-467.

Gámiz-Gracia, L., A.M. García-Campaña, J.F. Huertas-Pérez and F. J. Lara (2009). Chemiluminescence detection in liquid chromatography: Applications to clinical, pharmaceutical, environmental and food analysis–A review. Analytica Chimica Acta, 640: 7-28.

Gauglitz, G. and T. Vo-Dinh (2003). Handbook of Spectroscopy. Published by: WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Federal Republic of Germany.

Gómez-Alonso, S., V. Mancebo-Campos, M. D. Salvador and G. Fregapane (2007). Evolution of major and minor components and oxidation indices of virgin olive oil during 21 months storage at room temperature. Food Chemistry, 100: 36-42.

Gómez-Ariza, J.L., A. Arias-Borrego, T. García-Barrera and R. Beltran (2006). Comparative study of electrospray and photospray ionization sources coupled to quadrupole time-of-flight mass spectrometer for olive oil authentication. Talanta, 70: 859-869.

Gordon, M.H., C. Covell and N. Kirsch (2001). Detection of pressed hazelnut oil in admixtures with virgin olive oil by analysis of polar components. Journal of the American Oil Chemists Society, 78: 621-624.

Guadarrama, A., M.L. Rodríguez-Méndez, C. Sanz, J.L. Ríos and J.A. De Saja (2001). Electronic nose based on conducting polymers for the quality control of the olive oil aroma: Discrimination of quality, variety of olive and geographic origin. Analytica Chimica Acta, 432: 283-292.

Guillén, M.D. and A. Ruiz (2001). High resolution ^{1}H nuclear magnetic resonance in the study of edible oils and fats. Trends in Food Science & Technology, 12: 328–338.

Guillén, M.D. and N. Cabo (1997). Infrared spectroscopy in the study of edible oils and fats. Journal of the Science of Food and Agriculture, 75: 1–11.

Guillén, M.D. and N. Cabo (1999). Usefulness of the frequencies of some Fourier transform infrared spectroscopic bands for evaluating the composition of edible oil mixtures. Lipid/Fett, 101: 71–76.

Guillén, M.D. and N. Cabo (2002). Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. Food Chemistry, 77: 503 – 510.

Guimet, F., J. Ferré, R. Boqué and F.X. Rius (2004). Application of unfold principal component analysis and parallel factor analysis to the exploratory analysis of olive oils by means of excitation-emission matrix fluorescence spectroscopy. Analytica Chimica Acta, 515: 75-85.

Guimet, F., J. Ferré and R. Boqué (2005). Rapid detection of olive–pomace oil adulteration in extra virgin olive oils from the protected denomination of origin “Siurana” using excitation–emission fluorescence spectroscopy and three-way methods of analysis. Analytica Chimica Acta, 544: 143-152.

Gurdeniz, G. and B. Ozen (2009). Detection of adulteration of extra-virgin olive oil by chemometric analysis of mid-infrared spectral data. Food Chemistry, 116: 519-525.

Gutiérrez, F., M.J. Villafranca and J.M. Castellano (2002). Changes in the main components and quality indices of virgin olive oil during oxidation. Journal of the American Oil Chemists Society, 79: 669-676.

Haddada, F.M., H. Manai, D. Daoud, X. Fernandez, L. Lizzani-Cuvelier and M. Zarrouk (2007). Profiles of volatile compounds from some monovarietal Tunisian virgin olive oils. Comparison with French PDO. Food Chemistry, 103: 467-476.

Hajimahmoodi, M., Y.V. Heyden, N. Sadeghi, B. Jannat, M.R. Oveisi and S. Shahbazian (2005). Gas-chromatographic fatty acid fingerprints and partial least squares modeling as a basis for the simultaneous determination of edible oil mixtures. Talanta, 66: 1108–1116.

Hendl, O., J.A. Howell, J. Lowery and W. Jones (2001). A rapid and simple method for the determination of iodine values using derivative Fourier transform infrared measurements. Analytica Chimica Acta, 427: 75–81.

Huang, C.L. and B.E. Sumpio (2008). Olive oil, the Mediterranean diet, and cardiovascular health. Journal of American College of Surgeons, 207: 407-416.

Iñón, F.A., J.M. Garrigues, S. Garrigues, A. Molina and M. De La Guardia (2003). Selection of calibration set samples in determination of olive oil acidity by partial least squares–attenuated total reflectance–Fourier transform infrared spectroscopy. Analytica Chimica Acta, 489: 59-75.

IOOC (2001). International Olive Oil Council. Preparation of fatty acid methyl esters from olive oils and olive-pomace oils. COI/T20/Doc. no. 24. Available online at http://www.internationaloliveoil.org.

IOOC (2008). International Olive Oil Council. Trade standard applying to olive oils and olive-pomace oils. COI/T.15/NC no. 3/Rev.3. Available online at http://www.internationaloliveoil.org.

Ismael, A.I. (1989). Effect of vegetable oils components on oils stability, Ph.D. Thesis, Fac. of Agric., Cairo Univ.

Jakab, A., K. Héberger and E. Forgács (2002). Comparative analysis of different plant oils by high performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography A, 976: 255–263.

Kapoulas, V.M. and N.K. Andrikopoulos (1987). Detection of virgin olive oil adulteration with refined oils by second-derivative spectrophotometry. Food Chemistry, 23: 183-192.

Kates, N. (1972). Technique of Lipidology, Elsevier Publishing Co. Inc.

Khalil, M.F. (1987). Evaluation of butter fat and virgin olive oil quality and identity, Ph.D. Thesis, Fac. of Agric., Cairo Univ.

Khalil, M.F., El-Sharkawy, A.A., Hallabo, S.A. and Morsi, M.K. (1983). Effect of some technological treatments in Shemlali olive oil. First African Conference of Food Science and Technology, Nov., 1983, Cairo, Egypt, 271-285.

Koprivnjak, O., D. Škevin, S. Valić, V. Majetić, S. Petričević and I. Ljubenkov (2008). The antioxidant capacity and oxidative stability of virgin olive oil enriched with phospholipids. Food Chemistry, 111: 121-126.

Lagarda, M.J., G. García-Llatas and R. Farré (2006). Analysis of phytosterols in foods. Journal of Pharmaceutical and Biomedical Analysis, 41: 1486–1496.

Lai, Y.W., E.K. Kemsley and R.H. Wilson (1995). Quantitative analysis of potential adulterants of extra virgin olive oil using infrared spectroscopy. Food Chemistry, 53: 95-98.

Lorenzo, I.M., J.L.P. Pavón, M.E.F. Laespada, C.G. Pinto and B.M. Cordero (2002). Detection of adulterants in olive oil by headspace–mass spectrometry. Journal of Chromatography A, Volume 945: 221-230.

Maggio, R.M., T.S. Kaufman, M.D. Carlo, L. Cerretani, A. Bendini, A. Cichelli and D. Compagnone (2009). Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infrared spectroscopy coupled with partial least squares. Food Chemistry, 114: 1549-1554.

Manai, H., F. Mahjoub-Haddada, I. Oueslati, D. Daoud and M. Zarrouk (2008). Characterization of monovarietal virgin olive oils from six crossing varieties. Scientia Horticulturae, 115: 252-260.

Manral, M., M.C. Pandey, K. Jayathilakan, K. Radhakrishna and A.S. Bawa (2008). Effect of fish *(Catla catla)* frying on the quality characteristics of sunflower oil. Food Chemistry, 106: 634-639.

Marini, F., F. Balestrieri, R. Bucci, A.D. Magrì, A.L. Magrì and D. Marini (2006). Class-modeling techniques in the authentication of Italian oils from Sicily with a Protected Denomination of Origin (PDO). Chemometrics and Intelligent Laboratory Systems, 80: 140–149.

Marini, F., F. Balestrieri, R. Bucci, A.D. Magrì, A.L. Magrì and D. Marini (2004). Supervised pattern recognition to authenticate Italian extra virgin olive oil varieties. Chemometrics and Intelligent Laboratory Systems, 73: 85–93.

Matos, L.C., S.C. Cunha, J.S. Amaral, J.A. Pereira, P.B. Andrade, R.M. Seabra and B.P.P. Oliveira (2007). Chemometric characterization of three varietal olive oils (Cvs. Cobrançosa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices. Food Chemistry, 102: 406–414.

Mavromoustakos, T., M. Zervou, G. Bonas, A. Kolocouris and P. Petrakis (2000). A novel analytical method to detect adulteration of virgin olive oil by other oils. Journal of the American Oil Chemists Society, 77: 405-411.

Méndez, A.I. and E. Falqué (2007). Effect of storage time and container type on the quality of extra-virgin olive oil. Food Control, 18: 521-529.

Mildner-Szkudlarz, S. and H.H. Jeleń (2008). The potential of different techniques for volatile compounds analysis coupled with PCA for the detection of the adulteration of olive oil with hazelnut oil. Food Chemistry, 110: 751-761.

Mohamed, D.M. (1999). Biochemical changes in oils during some industrial operations, Ph.D. Thesis, Fac. of Agric., Cairo Univ.

Morelló, J.R., M.J. Motilva, M.J. Tovar and M.P. Romero (2004). Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry, 85: 357-364.

Moros, J., M. Roth, S. Garrigues, and M. de La Guardia (2009). Preliminary studies about thermal degradation of edible oils through attenuated total reflectance mid-infrared spectrometry. Food Chemistry, 114: 1529–1536.

Muik, B., B. Lendl, A. Molina-Diaz, M. Valcarcel and M. J. Ayora-Cañada (2007). Two-dimensional correlation spectroscopy and multivariate curve resolution for the study of lipid oxidation in edible oils monitored by FTIR and FT Raman spectroscopy. Analytica Chimica Acta, 593: 54-67.

Nagy, K., D. Bongiorno, G. Avellone, P. Agozzino, L. Ceraulo and K. Vékey (2005). High performance liquid chromatography–mass spectrometry based chemometric characterization of olive oils. Journal of Chromatography A, 1078: 90-97.

Najafian, L., A. Ghodsvali, M.H.H. Khodaparast and L.L. Diosady (2009). Aqueous extraction of virgin olive oil using industrial enzymes. Food Research International, 42: 171-175.

National Sunflower Association (2009). Sunflower Oil. Trading Rules, Specifications from American Fats and Oils Association. Available online at http://www.sunflowernsa.com.

Ninfali, P., M. Bacchiocca, E. Biagiotti, M. Servili and G. Montedoro (2002). Validation of the oxygen radical absorbance capacity (ORAC) parameter as a new index of quality and stability of virgin olive oil. Journal of the American Oil Chemists Society, 79: 977-982.

Ocakoglu, D., F. Tokatli, B. Ozen and F. Korel (2009). Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. Food Chemistry, 113: 401-410.

Ollivier, D., J. Artaud, C. Pinatel, J.P. Durbec and M. Guérère (2006). Differentiation of French virgin olive oil RDOs by sensory characteristics, fatty acid and triacylglycerol compositions and chemometrics. Food Chemistry, 97: 382–393.

Oueslati, I., C. Anniva, D. Daoud, M.Z. Tsimidou and M. Zarrouk (2009). Virgin olive oil (VOO) production in Tunisia: The commercial potential of the major olive varieties from the arid Tataouine zone. Food Chemistry, 112: 733-741.

Őzdemir, D. and B. Őztűrk (2007). Near-infrared spectroscopic determination of olive oil adulteration with sunflower and corn Oil. Journal of Food and Drug Analysis, 15: 40-47.

Papadopoulous, K., T. Triantis, C.H. Tzikis, A. Nikokavoura and D. Dimotikali (2002). Investigation of the adulteration of extra virgin olive oils with seed oils using their weak chemiluminescence. Analytica Chimica Acta, 464: 135-140.

Pardo, J.E., M.A. Cuesta and A. Alvarruiz (2007). Evaluation of potential and real quality of virgin olive oil from the designation of origin ''Aceite Campo de Montiel'' (Ciudad Real, Spain). Food Chemistry, 100: 977–984.

Park, J.R. and D.S. Lee (2003). Detection of adulteration in olive oils using triacylglycerols compositions by high temperature gas chromatography. Bulletin of Korean Chemical Society, 24: 527-530.

Peña, F., S. Cárdenas, M. Gallego and M. Valcárcel (2005). Direct olive oil authentication: Detection of adulteration of olive oil with hazelnut oil by direct coupling of headspace and mass spectrometry, and multivariate regression techniques. Journal of Chromatography A, 1074: 215-221.

Peris, M. and L. Escuder-Gilabert (2009). A 21st century technique for food control: Electronic noses. Analytica Chimica Acta, 638: 1-15.

Phillips, K.M., D.M. Ruggio, J.I. Toivo, M.A. Swank and A.H. Simpkins (2002). Free and esterified sterol composition of edible oils and fats. Journal of Food Composition and Analysis, 15: 123-142.

Pinelli, P., C. Galardi, N. Mulinacci, F. F. Vincieri, A. Cimato and A. Romani (2003). Minor polar compound and fatty acid analyses in monocultivar virgin olive oils from Tuscany. Food Chemistry, 80: 331-336.

Poulli, K.I., G.A. Mousdis and C.A. Georgiou (2007). Rapid synchronous fluorescence method for virgin olive oil adulteration assessment. Food Chemistry, 105: 369-375.

Publio, V. and V. Marzia (2009). Virgin olive oil as a fundamental nutritional component and skin protector. Clinics in Dermatology, 27: 159-165.

Quiles, J.L., M.C. Ramírez-Tortosa, J.A. Gómez, J.R. Huertas and J. Mataix (2002). Role of vitamin E and phenolic compounds in the antioxidant capacity, measured by ESR, of virgin olive, olive and sunflower oils after frying. Food Chemistry, 76: 461-468.

Runcio, A., L. Sorgonà, A. Mincione, S. Santacaterina and M. Poiana (2008). Volatile compounds of virgin olive oil obtained from Italian cultivars grown in Calabria. Effect of processing methods, cultivar, stone removal, and antracnose attack. Food Chemistry, 106: 735-740.

Russin, T.A., F.R. van de Voort and J. Sedman (2003). Novel method for rapid monitoring of lipid oxidation by FTIR spectroscopy using disposable IR cards. Journal of the American Oil Chemists Society, 80: 635-641.

Saitta, M., F. Salvo, G. Di Bella, G. Dugo and G.L. La Torre (2009). Minor compounds in the phenolic fraction of virgin olive oils. Food Chemistry, 112: 525-532.

Salvador, M.D., F. Aranda, S. Gómez-Alonso and G. Fregapane (2001). Cornicabra virgin olive oil: a study of five crop seasons. Composition, quality and oxidative stability. Food Chemistry, 74: 267-274.

Salvador, M.D., F. Aranda, S. Gómez-Alonso and G. Fregapane (2003). Influence of extraction system, production year and area on Cornicabra virgin olive oil: a study of five crop seasons. Food Chemistry, 80: 359-366.

Sánchez-Gimeno, A.C., A.I. Negueruela, M. Benito, A. Vercet and R. Oria (2008). Some physical changes in Bajo Aragón extra virgin olive oil during the frying process. Food Chemistry, 110: 654-658.

Schulz, H. and M. Baranska (2007). Identification and quantification of valuable plant substances by IR and Raman spectroscopy. Vibrational Spectroscopy, 43: 13–25.

Sherazi, S.T.H., A. Kandhro, S.A. Mahesar, M.I. Bhanger, M.Y. Talpur and S. Arain (2009). Application of transmission FT-IR spectroscopy for the *trans* fat determination in the industrially processed edible oils. Food Chemistry, 114: 323–327.

Sikorska, E., T. Górecki, I.V. Khmelinskii, M. Sikorski and J. Kozioł (2005). Classification of edible oils using synchronous scanning fluorescence spectroscopy. Food Chemistry, 89: 217–225.

Sinelli, N., M.S. Cosio, C. Gigliotti and E. Casiraghi (2007). Preliminary study on application of mid infrared spectroscopy for the evaluation of the virgin olive oil “Freshness”. Analytica Chimica Acta, 598: 128–134.

Smith, S.A., R.E. King and D.B.Min (2007). Oxidative and thermal stabilities of genetically modified high oleic sunflower oil. Food Chemistry, 102: 1208-1213.

Tasioula-Margari, M. and O. Okogeri (2001). Simultaneous determination of phenolic compounds and tocopherols in virgin olive oil using HPLC and UV detection. Food Chemistry, 74: 377-383.

Tay, A., R.K. Singh, S.S. Krishnan and J.P. Gore (2002). Authentication of olive oil adulterated with vegetable oils using Fourier transform infrared spectroscopy. Lebensmittel-Wissenschaft und-Technologie, 35: 99–103.

Torres, M.M. and D.M. Maestri (2006). The effects of genotype and extraction methods on chemical composition of virgin olive oils from Traslasierra Valley (Córdoba, Argentina). Food Chemistry, 96: 507-511.

Vaz-Freire, L.T., M.D.R. Gomes da Silva and A.M.C. Freitas (2009). Comprehensive two-dimensional gas chromatography for fingerprint pattern recognition in olive oils produced by two different techniques in Portuguese olive varieties Galega Vulgar, Cobrançosa e Carrasquenha. Analytica Chimica Acta, 633: 263-270.

Vigli, G., A. Philippidis, A. Spyros and P. Dais (2003). Classification of edible oils by employing ^{31}P and ^{1}H NMR spectroscopy in combination with multivariate statistical analysis. A proposal for the detection of seed oil adulteration in virgin olive oils. Journal of Agricultural and Food Chemistry, 51: 5715-5722.

Vittadini, E., J.H. Lee, N.G. Frega, D.B. Mina and Y. Vodovotz (2003). DSC determination of thermally oxidized olive oil. Journal of the American Oil Chemists Society, 80: 533-537.

Vlachos, N., Y. Skopelitis, M. Psaroudaki, V. Konstantinidou, A. Chatzilazarou and E. Tegou (2006). Applications of Fourier transform infrared spectroscopy to edible oils. Analytica Chimica Acta, 573,574: 459-465.

Vlahov, G. (2006). ^{13}C nuclear magnetic resonance spectroscopy to determine olive oil grades. Analytica Chimica Acta, 577: 281-287.

Wesley, I.J., F. Pacheco and A.E.J. McGill (1996). Identification of adulterants in olive oils. Journal of the American Oil Chemists Society, 73: 515-518.

Wesley, I.J., R.J. Barnes and A.E.J. McGill (1995). Measurement of adulteration of olive oils by near-infrared spectroscopy. Journal of the American Oil Chemists Society, 72: 289-292.

Yang, D.P., D.X. Kong and H.Y. Zhang (2007). Multiple pharmacological effects of olive oil phenols. Food Chemistry, 104: 1269-1271.

Yang, H. and J. Irudayaraj (2001). Comparison of near-infrared, Fourier transform-infrared, and Fourier transform-Raman methods for determining olive-pomace oil adulteration in extra virgin olive oil. Journal of the American Oil Chemists Society, 78: 889-895.

Yang, H., J. Irudayaraj and M.M. Paradkar (2005). Discriminant analysis of edible oils and fats by FTIR, FT-NIR and FT-Raman spectroscopy. Food Chemistry, 93: 25–32.

Zabaras, D. and M.H. Gordon (2004). Detection of pressed hazelnut oil in virgin olive oil by analysis of polar components: improvement and validation of the method. Food Chemistry, 84: 475-483.

الملخص العربي

لقد ازدادت أهمية الكشف عن الغش في زيت الزيتون البكر الممتاز بتزايد اهتمام الرأي العام بأساليب غش زيت الزيتون بخلطه بالزيوت الأقل جودة وثمناً مثل زيوت عباد الشمس، فول الصويا، السمسم، الذرة أو بخلطه بزيوت الزيتون الأقل جودة، خاصة بعد الاكتشافات الطبية التي لا تحصي والتي أثبتت فوائد زيت الزيتون. ولا شك أن أهمية زيت الزيتون البكر الممتاز وارتفاع ثمنه جعل منه سلعة نفيسة قد تغري بغشها بالزيوت الأقل جودة والأرخص ثمناً لتحقيق أرباح طائلة، وذلك وإن كان يمثل إهدار لقيمة زيت الزيتون البكر الممتاز فإنه أيضاً قد تقل أو تنعدم فوائده التي يطلبها من يشتريه.

ومن هنا جاءت فكرة هذا البحث الذي يهدف في المقام الأول إلى خدمة المجتمع وإظهار ألاعيب من يقومون بالغش وذلك بأسلوب علمي مبسط قادر على مخاطبة المستهلك العادي حتى يأخذ حذره ويستطيع معرفة جودة الزيت النقي.

ولقد ساعد التطور المستمر في طرق التحليل المختلفة وبخاصة التحليل الطيفي بالمنطقة تحت الحمراء في مجال بحوث الأغذية على تيسير طرق كشف الغش في زيت الزيتون البكر الممتاز.

ويبدأ هذا البحث بتصميم خلطات ثنائية من زيت الزيتون البكر الممتاز مع زيوت الغش الشائعة مثل زيت عباد الشمس، الذرة، والزيتون المكرر منخفض الجودة، حيث تم الحصول على زيت الزيتون البكر الممتاز باستخلاصه بالطرق المعتمدة من ثمار الزيتون الطازجة في موسم حصاده. أما الزيوت الباقية فقد تم شراءها من السوق المحلي. وتم تحضير خلطات (وزن/وزن) مشابهة لخلطات الغش وذلك بنسب وزنية محددة (5، 10، 20، 30، 40، 50، 100%).

وأجريت التحاليل الطبيعية والكيميائية للزيوت النقية والمخاليط وهي:

معامل الإنكسار، رقم ونسبة الحموضة، رقم البيروكسيد والرقم اليودي. كما أجريت التقديرات الطيفية بالأشعة فوق البنفسجية عند الأطوال الموجية 232 ، 270 نانومتر وكذلك عند الأطوال الموجية 262 ، 268 ، 274 نانومتر لحساب ΔK. بالإضافة إلى إجراء التحليل الكروماتوجرافي الغازي لتقدير الأحماض الدهنية، كما قدرت المواد الكلية غير القابلة للتصبن؛ كذلك أجري تقدير لطيف امتصاص الزيوت النقية والمخاليط بمنطقة الأشعة تحت الحمراء باستخدام جهاز التحليل الطيفي، وذلك للتوصل إلى الخواص المميزة للزيوت النقية والمخاليط المحضرة من زيت الزيتون البكر مع الزيوت المستخدمة للغش بتركيزات مختلفة للتوصل إلى طرق كشف هذا الغش. كما أجريت حسابات وعلاقات على نتائج تحليل الأحماض الدهنية وكذلك نتائج تقدير طيف الامتصاص بمنطقة الأشعة تحت الحمراء.

وفيما يلي ملخص لأهم النتائج المتحصل عليها:

ـ أفاد معامل الانكسار في كشف الغش بزيت عباد الشمس فقد أعطي قراءات خارج المدي المحدد بالمواصفات القياسية لزيت الزيتون عند تقديره في المخاليط التي يزيد تركيزها عن 30% منه. وكانت حساسيته للكشف عن الغش بزيت الذرة عندما يكون تركيزه 20%. أما زيت الزيتون المكرر فإنه لم يظهر أي دليل على الغش بهذا الزيت وأعطي قراءات داخل المدي المنصوص عليه بالمواصفات القياسية.

ـ عند تقدير درجة الحموضة فإنها لم تظهر أي دليل على الغش.

ـ وقد وجد أيضاً عند تقدير رقم البيروكسيد أنه لم يظهر أي دلالة على غش زيت الزيتون البكر بالزيوت الأخرى تحت الدراسة.

ـ ودلت النتائج على أن تقدير الرقم اليودي قد أفاد في كشف الغش بزيت عباد الشمس وأعطي قراءات خارج المدي المحدد بالمواصفات القياسية لزيت الزيتون عند تقديره في المخاليط التي يزيد تركيزها عن 30% في زيت الزيتون وكذلك أعطي حساسية للكشف في زيت الذرة عند تقديره في المخاليط التي يزيد تركيزها عن 30% من زيت الذرة مع زيت الزيتون. أما الغش بزيت الزيتون المكرر فإنه لم يظهر أي دليل على الغش حيث أعطي قراءات داخل المدي المنصوص عليه بالمواصفات القياسية.

ـ عند إجراء التقديرات الطيفية للمخاليط بالأشعة فوق البنفسجية, حيث أظهرت خواص مميزة لها عند الطول الموجي 232 نانومتر والذي يميز وجود الرابطة الثنائية المتبادلة وكذلك أجريت التقديرات الطيفية للمخاليط عن الطول الموجي 270 نانومتر والذي يميز وجود الرابطة الثلاثية المتبادلة وقد أظهر التحليل الطيفي بالمنطقة فوق البنفسجية عند الطول الموجي 270 نانومتر دليل على الغش ابتداء من تركيز 20% من زيت عباد الشمس وعند اجراء هذا التحليل على مخاليط زيت الذرة مع زيت الزيتون فقد أظهر نتائج ممتازة فقد أمكن كشف الغش عند وجود زيت الذرة بنسبة 5% فقط. كما أمكن كشف الغش بزيت الزيتون المكرر عند إضافته بنسبة 10% وذلك حيث كانت القيم المسجلة خارج نطاق القيم القياسية المسجلة بالمواصفات القياسية.

ـ عند تقدير معدل التغير في الامتصاص عند الأطوال الموجية المختلفة وحساب ΔK أظهرت النتائج إمكانية كشف الغش بزيت عباد الشمس عند وجوده بنسبة 5%، كذلك أمكن كشف الغش بزيت الذرة أيضاً عند وجوده بنسبة 5%. أما زيت الزيتون المكرر فإن أقل تركيز منه يمكن كشفه في الغش كان 10% في المخلوط مع الزيت النقي.

ـ عند اجراء التحليل الكروماتوجرافي الغازي للكشف عن الأحماض الدهنية في المخاليط الثنائية لزيت الزيتون مع الزيوت المضافة بنسب مختلفة قد أمكن كشف الغش بتحليل الأحماض الدهنية وكان الحامض الدهني المميز لكشف الغش هو حامض اللينولييك عند وجوده في المخلوط المغشوش بزيت عباد الشمس ابتداءً من تركيز 30% منه. أما زيت الذرة المستخدم في الغش فقد أمكن الكشف عنه أيضاً عن طريق حامض اللينولييك عند وجود زيت الذرة بنسبة تبدأ من 30% منه. في حين أمكن الكشف عن زيت الزيتون المكرر المستخدم في الغش بالكشف عن حامض حامض اللينولينيك ابتداءً من تركيز 20% منه.

ـ عند تقدير طيف امتصاص المخاليط بمنطقة الأشعة تحت الحمراء باستخدام جهاز التحليل الطيفي بالمنطقة تحت الحمراء تم تسجيل بيانات التحليل الطيفي بمنطقة الأشعة تحت الحمراء في المدى من 4000 – 400 سم$^{-1}$ لعينات المخاليط التي تم إدخالها للجهاز في صورتها بدون أي تعديلات أو تحويلات أو طرق استخلاص واشتقاق وذلك من مميزات التقدير الطيفي بالأشعة تحت الحمراء حيث لا يتطلب أي مجهود أو تحضيرات أو وقت. وكانت النتائج متميزة حيث لوحظ وجود انحراف طيفي عند القيمة 3005 سم$^{-1}$ (المميز لوجود رابطة CH= المطاطية الاهتزاز والزوجية في الوضع *cis*– المميزة لزيت الزيتون) لقيم أعلي ؛ وذلك في مخاليط الغش بزيت عباد الشمس وزيت الذرة مما ساعد على كشف الغش بهما ولم تلاحظ في مخاليط الغش زيت الزيتون المكرر.

ـ كذلك ظهرت نتائج متميزة في المدى الطيفي 1300 – 1000 سم$^{-1}$ والتي اعتبرت كبصمة الإصبع في الزيوت، حيث أظهرت القيمة 1163 سم$^{-1}$ (رابطة C–O المطاطية الاهتزاز ورابطة C–H الإلتوائية الاهتزاز) زيادة في شدة الامتصاص عند هذه النقطة بازدياد معدلات الغش في زيت الزيتون وكانت نسبتا الامتصاص (R 1097/1118 سم$^{-1}$) و (R 2925/1747 سم$^{-1}$) مفيدة جداً حيث سجلت قيمهما انخفاضات بتزايد نسبة الغش.

ـ كما ظهرت إزاحة واضحة لمنحنى الرابطة (HC=CH) عند القيمة 912 سم$^{-1}$ نتيجة للالتواء الحادث في الرابطة خارج المستوي وهذه الإزاحة تزداد بزيادة نسبة الغش.

ـ وهكذا تتضح مدي دقة وسهولة وسرعة تحليل الطيف في منطقة الأشعة تحت الحمراء في كشف الغش في زيت الزيتون البكر الممتاز.

جامعة عين شمس
كلية الزراعة

رسالة ماجستير

اسم الطالبة: رانيا إبراهيم محمد المصيلحي
عنوان الرسالة: تطبيق بعض طرق كشف الغش في زيت الزيتون
اسم الدَّرجة: ماجستير في العلوم الزراعية (علوم وتكنولوجيا الأغذية)

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صفحة الموافقة على الرسالة

تطبيق بعض طرق كشف الغش في زيت الزيتون

رسالة مقدمة من

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